

Nutritional Therapy of Endocrine Disturbances

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I. INTRODUCTION

Although direct nutritional therapy of endocrine disturbances is of very recent origin, numerous clinical observations during the past half century, viewed in retrospect, reveal surprising correlations between nutritional and endocrine disturbances. And scattered animal investigations during the past thirty years have indicated a more direct relation between nutritional deficiency and changes in endocrine function.

Among these older studies were the use of liver and liver extracts in diabetes (Gilbert and Carnot, 1896). Stockton (1908) described the characteristic appearance of the tongue in diabetes as "large, red, 'beefy,' and bordered with a fissured margin . . .," a description now accepted as classical of pellagra. Functional changes in the central nervous system in diabetes that we now recognize as characteristic of thiamine and niacinamide deficiency were described with great accuracy by Futcher in 1907. The occurrence of hyperglycemia in pigeons on a "vitamin-free" diet was reported by Funk and von Schoenborn in 1914; this observation was followed up by other workers but its clinical implications were long neglected.

In chlorosis, long recognized as a nutritional disorder, menometrorrhagia and other menstrual disturbances were known to be characteristic (Cabot, 1908), and the occurrence of excessive uterine bleeding associated with cirrhosis of the liver, was equally well known three or four decades ago (Kelly, 1908; Rolleston, 1912). Goldberger subsequently observed menorrhagia in pellagra.

Plaut (1923) first reported hypertrophy of the adrenals in avitaminosis in animals.

Testicular atrophy occurring secondary to "malnutrition" was mentioned by Reynolds and Macomber in 1921, and both testicular atrophy and gynecomastia have been known for some time to be associated with hepatic cirrhosis (cited by Glass *et al.*, 1940).

More direct evidence of a relation between nutrition and endocrine function was provided by Evans and Bishop (1922); they reported that rats on a vitamin B free diet became anestrous. This phenomenon was later shown to be due to the suppression of the anterior pituitary (Parkes, 1928; Marrian and Parkes, 1929).

Despite these and other accumulated data, the concomitant appearance of nutritional and endocrine disturbances in the human being was considered to be accidental. Recent investigations have shown the nutritional defect, in many instances, to be etiologic in relation to the glandular dyscrasias. This will be discussed in the subsequent pages.

II. SYNDROMES RELATED TO EXCESS ESTROGEN

1. Relation of Nutritional Deficiency to Inactivation of Estrogen in the Liver

Soon after the isolation of theelin (Doisy, Veler and Thayer, 1929), it became apparent that this and related steroids are rapidly inactivated in the body (Frank, Goldberger and Spielman, 1932; Zondek, 1934). One earlier clue pointed to the possibility that estrogen is destroyed somewhere in the portal circulation. Evans and Burr (1926) had observed that estrogenic preparations were more effective when given subcutaneously than when administered intraperitoneally. The first suggestion that the liver might be a site of inactivation appears to have been made by Silberstein, Molnar and Engel (1933), although as Zondek (1935) pointed out, the method employed by these workers was not adequate to establish this conclusively. It was Zondek (1934) who first reported an extensive investigation of this problem. He demonstrated that the liver is capable of inactivating from 80 to 90 % of added estrogen *in vitro*. This function of the liver was subsequently confirmed in another way; circulation of estrone through a heart-lung perfusion system did not result in destruction of the estrogen but circulation through a heart-lung-liver system led to rapid inactivation (Israel, Meranze and Johnston, 1937). Estrus did not occur in animals in which the ovaries had been transplanted to the mesentery (in the portal circulation); regular estrous cycles did occur in rats having ovarian transplants in the axillae (Golden and Sevringshaus, 1938).

Employing the method of pellet implantation (Shelesnyak and Engle,

1932; Deanesly and Parkes, 1937), G. R. Biskind devised the technic of inserting pellets of crystalline steroids into the spleens of castrate male and female rats. With the spleens containing pellets in the normal situation in the portal circulation, no estrogenic or androgenic effect occurred with estrone, estradiol, estradiol benzoate, or with testosterone, testosterone propionate or methyl testosterone (G. R. Biskind and Mark, 1939; G. R. Biskind, 1940, 1941, 1942). Inactivation of both estrogens and androgens occurred in the livers of both male and female rats. If the spleen containing the pellet of steroid was subsequently transplanted subcutaneously and, after establishment of a collateral circulation, the pedicle was ligated, the specific estrogenic or androgenic effect became evident (G. R. Biskind and Mark, 1939).

On the basis of the latter investigations, and others implicating nutritional defects in alterations of hepatic morphology (Patek, 1937; Ando, 1938; Rhoads *et al.*, 1938, 1940; Sebrell and Onstatt, 1938; Nakahara *et al.*, 1939; György and Goldblatt, 1939, 1940; Rich and Hamilton, 1940) (*cf.* also: Blumberg and McCollum, 1941; Daft *et al.*, 1941; Earle *et al.*, 1941, 1942; Lillie *et al.*, 1941, 1942; Lowry *et al.*, 1941; Patek and Post, 1941; Broun and Muether, 1942), M. S. and G. R. Biskind (1941), utilizing the method of splenic implantation, found that, while castrate female rats with pellets of estrone in their spleens remained anestrous when on a normal diet, they went into continuous estrus when the diet was depleted in B complex vitamins, thus demonstrating that, in this type of nutritional deficiency, the liver loses its ability to inactivate estrogen. Addition of brewers yeast, or a mixture of crystalline thiamine, riboflavin, pyridoxine and calcium pantothenate, to the diet restored the anestrous state and subsequent depletion again led to continuous estrus (M. S. and G. R. Biskind, 1942; M. S. Biskind, 1943). Thus, the flow of estrogen through the liver could be controlled at will by withholding the B vitamins or restoring them to the diet. Impairment of the estrogen-inactivating mechanism of the liver occurred in the absence of detectable morphologic change in this organ (Fig. 1) (M. S. and G. R. Biskind, 1942); conversely, inactivation of estrogen can occur in livers which are the site of severe necrosis and fat infiltration, induced by a B complex-free diet supplemented with thiamine, riboflavin, pyridoxine and calcium pantothenate (Fig. 2) (M. S. Biskind, 1944). The functional and morphologic changes in the liver thus bear no necessary relation to each other.

Subsequent investigation of the problem of estrogen inactivation in the liver has shown that, *in the rat*, thiamine and riboflavin alone among the B vitamins are adequate to permit hepatic destruction of estrogen (Singher, Taylor *et al.*, 1944; Singher, Kensler *et al.*, 1944; Segaloff and Segaloff, 1944:

Shipley and György, 1944) and that the presence of methionine is essential to this function (Unna, Singher *et al.*, 1944; György and Goldblatt, 1945).*

* While this review was in press, Drill and Pfeiffer (1946) reported experiments from which they conclude, in contrast to the results obtained by the other investigators in this field, that "deficiency of the whole vitamin B complex affects the inactivation of estrogen only through the concomitant inanition produced," and that "supplements of methionine were without effect."

While it is important that the role of all nutrients in a given phenomenon be adequately assessed, in actual practice the tendency to dismiss the effects of the various vitamins as due to the associated inanition, has acted in the past greatly to retard proper evaluation of experimental data and to delay their application to human nutrition. The problems involved are complicated and the greatest care is necessary both in the performance of the investigations and in their interpretation. Reference may be made, for instance, to the observation of Funk and von Schoenborn (1914) on experimental diabetes and the subsequent work of Collazo (1922) and others, reviewed at length by Sherman and Smith (1931) and discussed briefly in Section V. Mason (1939) has reviewed the literature relating to the differential aspects of vitamin depletion and inanition on the reproductive apparatus, and Lepkovsky (1942) has pointed out (with specific reference to the effect of pantothenic acid on the testis) a serious pitfall common to virtually all paired feeding experiments used for separating the effects of specific vitamin deficiencies from those of the associated cachexia.

With specific reference to the aforementioned report of Drill and Pfeiffer, important discrepancies and omissions appear in relation to previously reported investigations in this field: In the experiments described by M. S. and G. R. Biskind (1941, 1942), estrual reactions occurred in some castrate animals with pellets of estrone in their spleens, as early as 2 or 3 days after being placed on a vitamin B complex-free diet, long before inanition could possibly be a factor. Shipley and György (1944) in observations on the same strain of rats, reported, "All became positive [estrous] within 5 to 14 days, and before any serious ill effects from the diet were evident." Estrual reactions as early as the third day of depletion also occurred in the experiments of Biskind and Shelesnyak (1942), in which one ovary was transplanted to the spleen and the other was removed. In addition, restoration of the B vitamins to the diet in severely depleted castrate animals with splenic pellets, led to resumption of the hepatic estrogen-inactivating function in as little as 2 to 4 days, before the cachectic state of the animals was significantly altered. Shipley and György observed this reversal under similar conditions, in as little as 3 or 4 days. Obviously, with respect to hepatic estrogen-inactivation in the animal with an intact gonad, inanition cannot be of practical importance, since, as is well known, ovarian estrogen secretion ceases during starvation, whereas Biskind and Shelesnyak showed that impairment of estrogen inactivation occurs long before ovarian function is depressed.

The prolonged period required to induce estrus in the experiments of Drill and Pfeiffer (20 to 30 days), compared to the experience of M. S. and G. R. Biskind and Shipley and György with the Sherman strain of rats (2 to 23 days in the former, 5 to 14 days in the latter) suggests that Drill and Pfeiffer may have used a relatively resistant strain (Shipley and György showed the Sherman variety to be the most susceptible to nutritional impairment of hepatic function of 3 strains tested). Unless the animals receiving a vitamin B complex-free diet are individually fed daily with fresh food (which was done in the original experiments of M. S. and G. R. Biskind), they stop eating long before they otherwise would. Because the utilization of

In connection with the previous study on the relation of B complex deficiency to the inactivation of estrogens, M. S. and G. R. Biskind (1943) investigated the effect of dietary depletion of the B vitamins on the inactivation of androgen in the liver. Unlike the estrogens, there was no *significant* impairment of the ability of the liver to destroy androgen in B complex deficiency. Thus, a serious alteration of the estrogen-androgen equilibrium must result. This has important clinical implications which will be discussed subsequently.

In view of the observation by Evans and Bishop (1922) that in vitamin B deficiency, rats become anestrous, M. S. Biskind and Shelesnyak (1942) studied the effect of vitamin B complex deficiency on rats in which one ovary had been removed and the other transplanted to the spleen. On a complete diet such animals remained anestrous; on a B complex-free diet all showed estrual reactions. Thus, it became apparent that the estrogen-inactivating mechanism of the liver can be impaired at a time when the ovary is still functional. This, too, has important clinical implications.

The observation by Plaut (1923), previously mentioned, that the adrenal cortex is hypertrophic in avitaminosis B in rats, is undoubtedly related to failure of inactivation of estrogen in the liver. Excess estrogen regularly produces this phenomenon (Korenchevsky and Dennison, 1935), and it was observed by the author in vitamin B deficient castrate animals with a pellet of estrogen or an ovary implanted in the spleen, showing protracted estrual reactions as a result of impaired inactivation in the liver.

thiamine and riboflavin is related to the caloric intake, failure to take this precaution may lead to inanition before the reserves of the B vitamins are depleted. It is not clear whether this precaution was taken by Drill and Pfeiffer. These workers kept their animals in the pre-depletion control period, not on a full diet, but on a purified diet plus A, D and 9 B factors; loss of other nutrients thus occurred even before intentional depletion (see Section VI).

Unna, Singher *et al.* (1944), György and Goldblatt (1945) and György (1945) have shown that, in the absence of an adequate amount of protein, the B vitamins cannot maintain estrogen-inactivation in the liver. Hence, restriction of diet as performed in the paired feeding experiments of Drill and Pfeiffer could easily have reduced the protein intake below the critical level, and the apparent failure of methionine in their experiments, in contrast to the positive effect reported by Unna *et al.* and György, may have been due (assuming the possibility that other factors were equal) to differences in the basic protein intake.

From unpublished experiments of G. R. and M. S. Biskind (interrupted by wartime exigencies) other factors in addition to thiamine, riboflavin and methionine appear to be involved in the estrogen-inactivating mechanism, and it appears that inanition can *also* disturb it, even though this has little practical significance. As discussed subsequently, in the clinical syndromes in which nutritional deficiency is associated with excess estrogen, the vast majority of the subjects are extremely well fed; the latter respond dramatically to administration of the whole B complex.

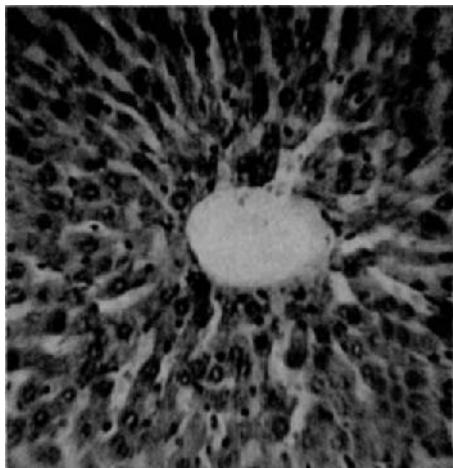


Fig. 1. Photomicrograph of liver of castrate female rat with pellet of estrone in the spleen, after depletion on a vitamin B complex-free diet. The morphology is normal although the liver had lost its estrogen inactivating function.

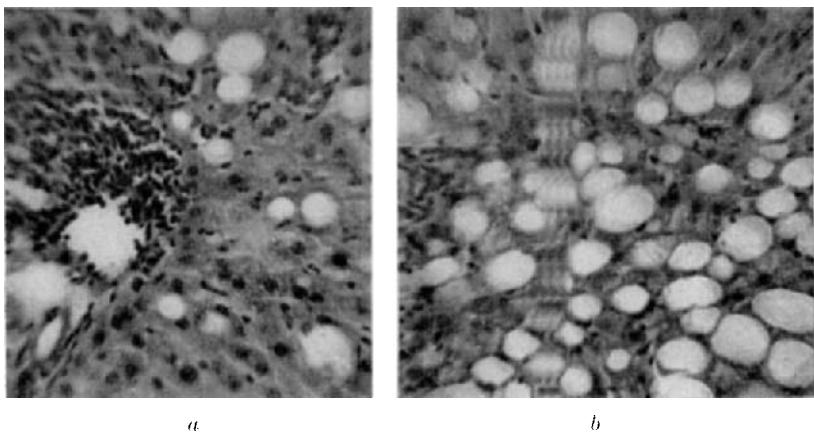


Fig. 2, *a* and *b*, Photomicrographs of livers of rats similar to animal represented in Fig. 1, except that thiamine, riboflavin, pyridoxine and pantothenic acid had been added to the vitamin B complex-free diet. The livers had regained their ability to inactivate estrone but are morphologically seriously damaged.

2. "Functional" Uterine Bleeding, Cystic Mastitis, Premenstrual Tension

Evidence relating the occurrence of certain forms of pathologic uterine bleeding and of premenstrual tension, chronic cystic mastitis and other disturbances to an excess of estrogen was provided originally by the fundamental work of R. T. Frank and his collaborators (Frank, 1931; Frank, Goldberger and Spielman, 1934; Frank, 1935; 1940). Ehrlich (1941) has

reported that the endometrium of patients with menorrhagia or metrorrhagia contains thrombotic phenomena similar to those illustrated by Zuckerman (1937) in the endometrium of castrated monkeys treated with estrogen.

The occurrence of excess estrogen in the clinical syndromes mentioned was thought to be due to excessive secretion by the ovaries (Frank, 1935). However, the observation that the liver fails to inactivate estrogen in vitamin B complex deficiency suggested another explanation (M. S. and G. R. Biskind, 1941, 1942, 1943; Biskind and Shelesnyak, 1942), and the relation of these syndromes to nutritional deficiency was therefore investigated (M. S. Biskind, 1943; M. S., G. R. and L. H. Biskind, 1943, 1944).

In three series of patients, involving at the time of writing, a total of more than 450 cases, a striking correlation was found between signs and symptoms of nutritional deficiency and the occurrence of syndromes related to excess estrogen. In these series, every patient, without exception, who had excessive uterine bleeding, cystic mastitis or premenstrual tension also had definite and usually severe objective and subjective indications of nutritional deficiency. Treatment with vitamin B complex orally, or orally and parenterally, produced rapid and dramatic improvement in the endocrine disturbances, along with healing of the avitaminotic lesions (see Figs. 3-6 and 8-10).

Among the patients with functional uterine bleeding, every endometrium examined showed evidence of estrogenic proliferation and the absence of a progesterone effect. The histologic appearance of the endometrium varied from that occurring in the early proliferative phase to cystic glandular hyperplasia and adenomyosis. Figs. 4, 5 and 6 illustrate the correlation of the lesions of nutritional deficiency and the estrogenic proliferation of the endometrium.

It has long been known that menorrhagia and metrorrhagia may occur early in the course of cirrhosis of the liver (Kelly, 1908; Rolleston, 1912). Excessive uterine bleeding has been reported also in intoxication with a number of liver poisons, such as lead, benzene, carbon disulfide and tetryl. Cirrhosis of the liver is now known, from the work of György and Goldblatt (1939, 1940, 1942) and others, to result from nutritional deficiency. Sources of the vitamin B complex have been shown to protect the liver against a variety of agents (such as lead, arsenic, carbon tetrachloride and dimethylaminoazobenzene) which cause functional and morphologic damage to this organ (Forbes and Neale, 1936; Forbes and McConnell, 1937; Von Glahn and Flinn, 1939; Ando, 1938; Nakahara *et al.*, 1939; Kensler *et al.*, 1940, 1941; Rhoads, 1940; Sugiura and Rhoads, 1941). In addition to this evidence, Goldberger (Goldberger and Sebrell, 1932) has reported that menorrhagia may occur in pellagra.

Glass, Edmondson and Soll (1940, 1944) have demonstrated in male patients with cirrhosis of the liver that the estrogen in the urine is increased and appears in active (uncombined) form, while the urinary androgen is somewhat reduced in amount and all of it continues to be excreted in combined form. These patients had gynecomastia, testicular atrophy, or both. These results agree precisely with the observation by M. S. and G. R. Biskind (1942, 1943) already mentioned, that in vitamin B complex deficiency the liver loses its ability to inactivate estrogen while it continues to inactivate androgen. Wu (1942) has observed histologic changes in the prostate glands of patients with cirrhosis of the liver, indicative of stimulation by estrogen. A survey of necropsy records of female patients with cirrhosis of the liver (M. S. and L. H. Biskind, 1943) showed evidence of excess estrogen in every case in which data were available on the pelvic organs.

It seems likely that dietary estrogen, which normally is destroyed in the liver, would largely or entirely escape inactivation in vitamin B complex deficiency and, added to endogenous estrogen, already in excess, would exert a further deleterious effect. That failure of inactivation of dietary estrogen occurs in cirrhosis of the liver is suggested in a case (for the report of which I am indebted to Dr. G. R. Biskind) of a woman aged 70, who died of hepatic cirrhosis; the endometrium showed extreme active cystic hyperplasia.

Bean (1942, 1943) has found that the cutaneous vascular spiders and palmar erythema formerly associated mainly with cirrhosis of the liver, occur also in nutritional deficiency and at the period in pregnancy when estrogen increases significantly. Administration of estrogen to patients with the cutaneous phenomena led to the appearance of new lesions and exacerbation of those already present; withdrawal of the estrogen caused regression of the vascular disturbances. Bean has correlated his observations with experimental studies by Reynolds and Foster (1940), who showed that estrogen causes dilatation of the minute vessels in the ears of the castrated rabbit, and he has shown that the cutaneous vascular spiders are histologically similar to the spiral arteries of the endometrium observed by Bartelmez and Markee (*cf.* Bartelmez, 1942) and by Jones and Brewer (1939). Perera (1942) has also reported the appearance of palmar erythema in nutritional deficiency. He noted regression of this lesion in two cases of hepatic cirrhosis under dietary and vitamin therapy.

A considerable proportion of patients who have lesions of nutritional deficiency associated with functional uterine bleeding, cystic mastitis and premenstrual tension also have the cutaneous vascular spiders and palmar erythema noted by Bean and by Perera. In addition, a tendency to develop petechial hemorrhages from relatively minor bruises and an increased tend-

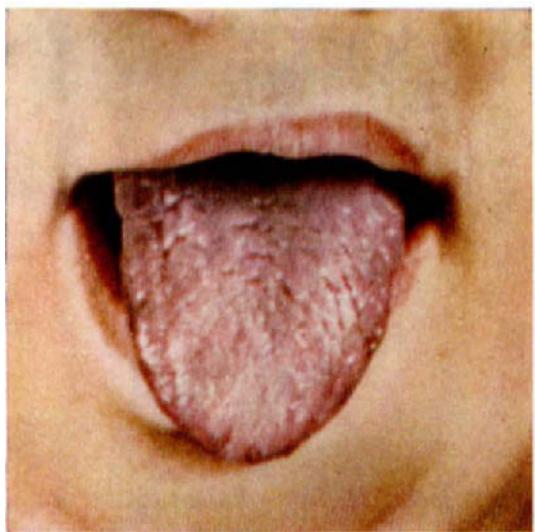


FIG. 3 *a*



FIG. 4 *a*

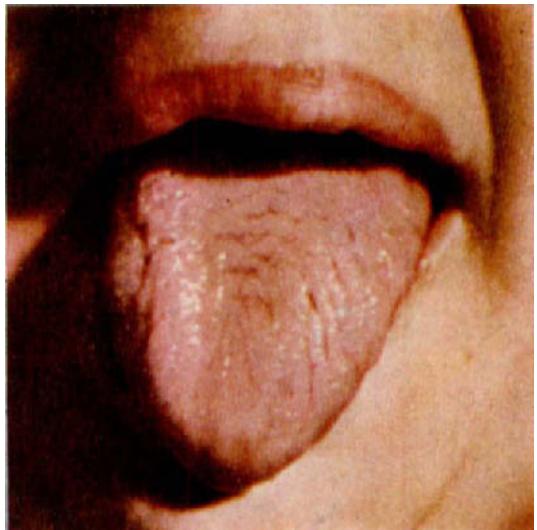


FIG. 3 *b*



FIG. 4 *b*

Fig. 3. *a*, Atrophic glossitis in a patient with cystic mastitis and premenstrual tension; following a pregnancy two years previously she had had subinvolution of the uterus and menometrorrhagia. *b*, Healing of the glossitis after treatment with vitamin B complex; the cystic mastitis and premenstrual tension completely cleared up. Both the glossitis and the premenstrual symptoms recurred as soon as dosage of the B complex was reduced below the maintenance level.

Fig. 4. *a*, Atrophic glossitis observed in a patient immediately following hysterectomy for uterine myomas and menometrorrhagia. *b*, Cystic hyperplasia of the endometrium in hysterectomy specimen.

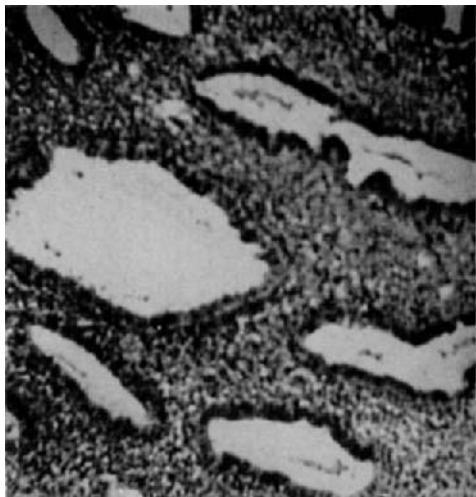
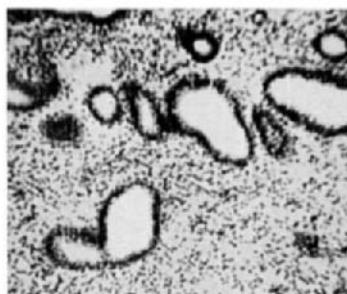
FIG. 5 *b*FIG. 6 *a*FIG. 6 *b*

Fig. 5. *a*, Atrophic glossitis in a patient with menometrorrhagia. *b*, Glandular cystic hyperplasia of the endometrium in the same patient. The menometrorrhagia recurred after curettage; it responded promptly to vitamin B complex therapy.

Fig. 6. *a*, Atrophic glossitis in a patient with severe menometrorrhagia which recurred after curettage but responded promptly to vitamin B complex. *b*, Cystic hyperplasia of the endometrium in the same patient.

Fig. 7. Severe cheilosis and glossitis produced by administration of estrogen to a patient in tenuous nutritional equilibrium as the result of hyperthyroidism which had been treated by roentgen irradiation.



FIG. 5 a



FIG. 7

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ency to bleed, are extremely common among these patients. This clears up rapidly on vitamin B complex therapy (M. S. Biskind, 1943; M. S., G. R. and L. H. Biskind, 1944; M. S. Biskind, unpublished), indicating that this phenomenon is related to dilatation of cutaneous vessels under the influence of estrogen and is not due to vitamin K deficiency, which has been thought to bear a relation to menorrhagia (Gubner and Ungerleider, 1944). However, in the patients who have prominent cutaneous vascular spiders, these rarely show more than slight or moderate regression under treatment.* The palmar erythema, however, occasionally shows definite diminution after prolonged and intensive nutritional therapy (M. S. Biskind, unpublished).



FIG. 8 a



FIG. 8 b

Fig. 8. a. Central and marginal papillary atrophy of the tongue in a patient with menorrhagia, cystic mastitis and premenstrual tension. b. The effect of intensive, protracted therapy on the glossitis; the menstrual symptoms responded promptly to vitamin B complex, orally and parenterally. The glossitis healed slowly.

Deficiency of the vitamin B complex sets up a vicious cycle. For not only does vitamin B deprivation impair the inactivation of estrogen in the liver, but estrogen may cause vitamin B deficiency. Spontaneous exacerbations of signs of B deficiency occur during the menstrual cycle in relation to cyclic changes in body estrogen (Ashworth and Sutton, 1942; M. S. and G. R. Biskind, 1943; M. S. Biskind, 1943), and administration of estrogen leads to exacerbation of lesions already present. Fig. 7 shows the effect of administration of estrogen to a patient in tenuous nutritional equilibrium.

Heilig and Kantiengar (1942) found that in women in whom there is a relatively low liver function (as measured by the ability to convert benzoic to hippuric acid) on the 13th or 14th day of the menstrual cycle, there is a

* In a recent more extensive review, Bean (1945) has discussed factors, other than nutritional deficiency and the concomitant disturbance in estrogen metabolism, that may be involved in the production of the vascular spiders.

further diminution in liver function on the first day of menstruation. This suggests that in women who have impaired liver function as a result of vitamin B complex deficiency, there is further impairment during the period of the cycle when the highest level of estrogen occurs. A similar diminution in liver function occurs during the latter part of pregnancy (at the time when body estrogen rises to a high level) as measured by the excretion of administered bilirubin (Sullivan, Tew and Watson, 1934) or by the conversion of benzoic to hippuric acid (Hirsheimer, 1935). The latter observations are the more significant in that nutritional deficiency commonly



FIG. 9 a



FIG. 9 b

Fig. 9. a. Cheilosis, glossitis and characteristic "muddy" complexion in a patient who had had menorrhagia and severe premenstrual tension for 4 years. b. Effect of vitamin B complex therapy, oral and parenteral, on the avitaminotic lesions. Note partial healing of cheilosis and healing of glossitis and clearing of complexion. This patient had no menorrhagia or premenstrual tension for more than a year, so long as she continued intensive B complex therapy; these promptly recurred on reduction of dosage.

occurs in pregnancy, owing to the metabolic demands of the fetus which are usually not compensated by adequate fortification of the mother's diet (Tompkins, 1941; Williams and Fralin, 1942; Burke *et al.*, 1943). And menometrorrhagia, cystic mastitis and premenstrual tension frequently follow a pregnancy (M. S. Biskind, 1943).

Among the observations made in patients receiving vitamin B complex is that a number of patients who flowed usually for 5 or 6 days and had not considered this abnormal, subsequently flowed for not more than 3 or 4 days under therapy. This indicates that it may be necessary to revise our concept of the normal range in the duration of the menstrual flow.

Characteristically, patients with only mild or moderate signs or symptoms of vitamin B deficiency, who had become accustomed to increased nervous tension, insomnia, tenderness of the breasts, a feeling of "fullness" or "puffiness," lumbar backache, headache, increased fatigability, lower abdominal cramps and the like, as premonitory indications of impending

menstruation, reported after the first or second subsequent period while on B complex therapy, that the flow came on completely "without warning." Conversely, other patients, especially those who had signs of a more severe and more protracted deficiency and who were suffering from menorrhagia, reported that there had been dysmenorrhea for the first time while on vitamin B therapy, or that the pain had become more severe than before (this also occurred in one of the cases in which there were uterine myomas). In virtually every case in which this occurred there was little or no pain during subsequent periods while the nutritional therapy was continued.

The findings in animals indicating that the estrogen-androgen equilibrium is altered in B complex deficiency, is of especial interest in view of the response of the clinical conditions here discussed to treatment with androgens.



FIG. 10a



FIG. 10b

Fig. 10. *a*, Atrophic glossitis in a girl, aged 13½, with severe puberal bleeding since the menarche; bleeding occurred every 21 days, lasted 9 to 10 days. *b*, After one week on oral B complex therapy. A normal period occurred after 3 weeks of therapy. Normal periods then occurred every 27 days during the year subsequent that the patient was on therapy, under observation.

The therapeutic effect observed with the androgens is undoubtedly due to re-establishment of the estrogen-androgen equilibrium at a higher absolute level. Administration of the B complex, in contrast, reduces the body estrogen to the normal range and thus re-establishes the equilibrium at a physiologic level.

The use of estrogen in the (more or less successful) treatment of menorrhagia and of cystic mastitis appears paradoxical with reference to the observations indicating that these syndromes are caused by excess estrogen and respond to therapy which reduces the estrogen level. The occasionally successful use of estrogen in these cases does not alter the fact that this therapy is unphysiologic, accomplishing its end in menometrorrhagia probably by depressing or abolishing the cycle and maintaining a proliferative endometrium (which, in the absence of a cycle, may bleed little or not at

all). With reference to mastitis, Gardner (1941) has shown that while moderate doses of estrogen in castrated animals stimulate mammary growth, larger doses depress it. In any event, administration of estrogen in these cases not only does not cure the basic defect but actually makes it worse.

3. Postpartum Subinvolution of the Uterus

The generally accepted view with regard to postpartum subinvolution of the uterus, as expressed in current textbooks on obstetrics and gynecology, is that this condition results from local pathologic conditions in this



Fig. 11.

Atrophic glossitis in a patient with postpartum subinvolution of the uterus.

organ rather than from any systemic physiologic disturbance. The occurrence of incomplete uterine involution among patients having menometrorrhagia, cystic mastitis and premenstrual tension, associated with lesions of nutritional deficiency (see Fig. 11), suggested that the contrary might be the case (M. S. Biskind, 1943). On nutritional therapy in these patients, the uterus involuted rapidly and the endocrine disturbances cleared up promptly.

As postpartum uterine subinvolution appeared to be related to excess estrogen, owing to failure of destruction in the liver, L. H. and M. S. Biskind (1944) studied two groups of pregnant women. One was maintained on an average diet; the other received substantial supplements of vitamin B complex during pregnancy. All the patients were examined 6 weeks postpartum for evidence of uterine subinvolution. In the control group of 107, 6 patients had poor involution, in 23 it was fair, in 78 good and in none could it be called excellent. In the group of 76 that received B complex, none had poor involution; in 3 involution was fair, in 56 good and in 17 excellent,

—or satisfactory involution in approximately 96% of the women receiving B complex as against 73% in the control group. Thus, the rate of involution was definitely enhanced in the group receiving B complex.

Recent reports emphasize the inadequacies of American diets during pregnancy (Tompkins, 1941; Williams and Fralin, 1942; Burke, Beal *et al.*, 1943; Ebbs, 1943; Lockhart, Kirkwood and Harris, 1943; Bean, Spies and Blankenhorn, 1944). As L. H. and M. S. Biskind have pointed out, at times and in places that people subsisted largely on whole grains (Drummond and Wilbraham, 1939) they customarily required shorter periods of postpartum rest than our own usual minimum of ten days (Charles White, 1773; Goodell, 1875; Küstner, 1899—cited by Williams, 1927). This study provides further evidence for the need of greatly increased intake of vitamin B complex during pregnancy and the puerperium.

4. Diminished Libido and Impotence in the Male

As already mentioned, a number of isolated observations have appeared in the literature on impairment of gonadal function in the male, in conditions which we now know to be related, directly or indirectly, to nutritional deficiency, and in which disturbance of liver function occurs. For instance, in diabetes, T. B. Futcher wrote in 1907, "Loss of sexual desire and power in men is common, and may be an early feature." In intoxication with carbon disulfide, a liver poison, Edsall, writing the same year, pointed out that partial or complete impotence usually supervened. Cirrhosis of the liver has long been known to lead to testicular atrophy (cited by Glass *et al.*, 1940). Reynolds and Macomber (1921) noted the occurrence of testicular atrophy in "malnutrition."

In view of the alteration of the estrogen-androgen equilibrium which occurs through failure of inactivation of estrogen in the liver in vitamin B complex deficiency (M. S. and G. R. Biskind, 1943), (*cf.* also the observations of Glass *et al.*, and of Wu cited on page 154), it appeared likely that many cases of diminished libido and impotence in the male might be associated with nutritional deficiency, similar to the condition in the female. This was found to be the case (M. S. Biskind, 1944). There was a striking correlation between the occurrence of indications of nutritional deficiency (atrophic glossitis, cheilosis, gingivitis, seborrhea alae nasae, keratosis of the lower eyelids, conjunctival injection, cutaneous vascular spiders, emotional instability, insomnia, rapid fatigability, peripheral neuritis, *etc.*) and the presence of testicular softening and atrophy. Gynecomastia occurred occasionally. Not infrequently in these cases, the liver was large and tender. Under intensive vitamin B complex therapy, not only did the lesions of nutritional deficiency clear up (with rapid diminution in the size of the liver when this was enlarged) but there was rapid and dramatic restoration

of libido and potency. This was especially striking in cases of diabetes (Biskind and Schreier, 1945), in which diminished gonadal function in the male has long been considered almost invariable (*cf.* Root and Bailey, 1945).

Restoration of function in cases in which impotence is associated with nutritional deficiency is usually rapid and complete (of course, cases purely psychogenic in origin are excluded) with restoration of normal testicular texture but rarely of the original size (M. S. Biskind; Biskind and Schreier, 1945).

p-Aminobenzoic acid occasionally has been noted to produce marked increase in libido and potency (reviewed in Vol. II of *Vitamins and Hormones* by Ansbacher).

5. Implications for Industrial Toxicology

Numerous studies already cited have established that cirrhosis of the liver can be produced by nutritional deficiency or nutritional imbalance and that the B vitamins (especially choline) and the protein content of the diet play a major rôle in this phenomenon. In addition to the lesion just mentioned, other disturbances have been produced in the liver, both functional and morphological, by vitamin B deficiency (Rhoads and Miller, 1938; Sebrell and Onstatt, 1938). György and Goldblatt (1939, 1940, 1942) have shown that, in experimental cirrhosis of the liver in rats, the lesions may be prevented by the administration of brewer's yeast, yeast extract or choline. Subsequently, Blumberg and McCollum (1941) also reported prevention of dietary cirrhosis with choline, and Lowry and his co-workers (1941) have reported successful treatment of this condition with choline and casein. Patek (1937) and Patek and Post (1941), have noted amelioration of clinical hepatic cirrhosis with dietary therapy, mainly with sources of the vitamin B complex. Choline has also been found to be useful in clinical cirrhosis (Broun and Muether, 1941).

It has been demonstrated that the lesions produced by certain liver poisons resemble, at least in part, those which occur in nutritional deficiency (György and Goldblatt, 1939). Liver damage produced by carbon tetrachloride or chloroform may be prevented by liver extract (Forbes and Neale, 1936; Forbes and McConnell, 1937); that induced by lead arsenate may be prevented by brewer's yeast (Von Glahn and Flinn, 1939). Cirrhosis and carcinoma of the liver caused by dimethylaminoazobenzene ('butter yellow') may be prevented, in part or entirely, by liver extract, brewer's yeast, yeast extract, rice bran extract or by riboflavin and casein (Ando, 1938; Nakahara *et al.*, 1939; Rhoads, Kensler, Sugiura *et al.*, 1940, 1941; see also Burk and Winzler, 1944). Rhoads (1940, 1942) has pointed out that intoxication with butter yellow produces a secondary or 'conditioned' deficiency

of factors of the B complex. Rhoads and his co-workers have shown that breakdown products of butter yellow inhibit the cozymase system in very minute amounts.

Talbot (1939) extended the observation of Golden and Sevringshaus (1938) on destruction of endogenous estrogen by demonstrating that a liver poison, carbon tetrachloride, can impair the estrogen-inactivating mechanism. Administration of carbon tetrachloride to rats from 21 to 25 days old with intact ovaries led to a definite increase in the weight of the uterus over that of control animals. This effect did not occur in animals previously castrated. Pincus and Martin (1940) confirmed the observation that administration of carbon tetrachloride impairs the estrogen-inactivating system; the effectiveness of a given dose of estrone was thus increased about 80%.

These results are in agreement with the observations of Glass, Edmondson and Soll (Edmondson *et al.*, 1939; Glass *et al.*, 1940), already mentioned, on the excretion of free and combined estrogens and androgens in the urine of male patients with cirrhosis of the liver, all of whom had testicular atrophy, gynecomastia or both. They found that while all, or almost all, of the estrogen was excreted in active, uncombined form in these patients, free androgen was not found. Not only was there an absolute increase in the excretion of estrogen in these cases over the values for normal men but, in most of the cases, there was also a diminution in the excretion of androgen. The latter may have been due, at least in part, to depression of the gonadotropic function of the pituitary by the excess of free estrogen.

In view of the relation of nutritional deficiency to failure of inactivation of estrogen in the liver, discussed previously, and the extensive evidence that a variety of liver poisons act to produce a secondary deficiency of factors of the B complex, it might be expected that exposure to liver poisons in industry might lead to syndromes related to excess estrogen (M. S. and G. R. Biskind, 1942, 1943). Unfortunately, these syndromes have rarely been noted and, so far as the author can determine, have never been specifically investigated in relation to industrial toxicology. But in every case in which casual mention has been made of the menstrual function or of sexual potency in the male in relation to intoxication by liver poisons, the expected relationship appears. Thus, menorrhagia and metrorrhagia have been reported to occur in intoxication with lead (Edsall, 1907; Sollmann, 1942), benzene (Hamilton, 1926; International Labour Office, 1934), carbon disulfide (Edsall, 1907), tetryl ("nitramine") (Witkowski *et al.*, 1942); while exposure to lead (Sollmann, 1942) and carbon disulfide (Edsall, 1907; Braceland, 1942) are said also to cause loss of libido and impotence.

Another substance widely used in industry, furfural, was at one time sold as a nostrum for the treatment of amenorrhea ("Anogen," Notice of Judg-

ment, No. 27226, F. D. A., 1937). In rats on a polished rice diet Nakahara and Mori (1941) found that furfural produced hepatic cirrhosis. Feeding of furfural to castrate female rats with a pellet of estrone in the spleen, led to impairment of the estrogen-inactivating function of the liver in some animals even when the rats were maintained on a full diet (M. S. Biskind, unpublished).

Thus, the phenomenon of impaired inactivation of estrogen in the liver, whether induced by primary nutritional deficiency, by exposure to hepatotoxic agents or by other conditioning factors, appears to have extensive implications for industrial toxicology and for application of modern knowledge of nutrition among industrial workers. This problem requires extensive further investigation.

6. Prevention and Treatment of Neoplasms in Tissues Responsive to Estrogen

Investigations by many workers have shown that estrogen may be etiologically involved in the production of a variety of neoplasms in tissues responsive to estrogen, notably in the breast and uterus. The literature has been reviewed at length by Allen, Hisaw and Gardner (1939).

Clinical observations already described indicate a definite correlation between the occurrence of lesions of nutritional deficiency with impairment of the estrogen-inactivating function of the liver, and the incidence of lesions of the breast and myomas of the uterus (M. S. Biskind, 1943; M. S., G. R. and L. H. Biskind, 1944). Nutritional therapy has resulted not only in improvement in the functional conditions related to excess estrogen and in striking alleviation of cystic mastitis (considered by some investigators a pre-neoplastic lesion) but also in some cases in actual regression of fibroadenomas of the breast and myomas of the uterus (M. S., G. R. and L. H. Biskind, 1944; M. S. Biskind, unpublished).

Failure of the liver to inactivate estrogen in a deficiency of the vitamin B complex while this organ continues to inactivate androgen must seriously disturb the estrogen-androgen equilibrium. One possible consequence of such an alteration is indicated by the work of Lipschütz and his collaborators (1939-1944; cf. also Vargas, 1942; Marx *et al.*, 1942; Dosne, 1944; Iglesias *et al.*, 1944). Lipschütz *et al.* have shown that subserous fibroids can be produced by the continuous (but not by the intermittent) action of estrogen, not only in the uterus but also in other abdominal organs and in the abdominal wall. Fibroids thus produced can be prevented by the simultaneous administration of testosterone (or of progesterone or other steroids having the androstene nucleus).

When viewed in the light of present knowledge on the relation of the B vitamins to inactivation of estrogen in the liver, a number of otherwise

puzzling facts, especially in regard to tumors of the breast, become explicable. It is well known that the incidence of cancer of the breast is higher in obese women than in those of more nearly average proportions. This applies also to the occurrence of menorrhagia and other disturbances related to excess estrogen (M. S. Biskind, 1943). Loeb, Suntzeff *et al.* (1942) have shown that there is a definite direct correlation between body weight and the incidence of spontaneous mammary cancers in mice. The converse has been demonstrated by Tannenbaum (1940; 1942), who showed that caloric restriction diminished the incidence of mammary carcinomas.

As the need for thiamine and riboflavin is directly related to the caloric intake of carbohydrate, in a diet in which there is less than the minimal amount of these factors (and this is especially true of the present average American diet as well as that elsewhere) (Drummond and Wilbraham, 1939; Stiebeling, 1941, 1943; Jolliffe, 1943; Adamson *et al.*, 1945), the greater the caloric intake, especially of carbohydrate, the greater the vitamin deficit. Clinically, obese patients virtually always show signs of avitaminosis unless they have had a nutritional supplement.

The fact that diabetics show an incidence of cancer six times that of the general population as a whole (Ellinger and Landsman, 1944) is quite in keeping with the etiologic relationship of nutritional deficiency to diabetes (see Section V).

Minot (1938) has cited the classic case of a man who developed polyneuritis after gaining much weight on a high carbohydrate diet; the neuritis disappeared on dietary restriction without specific therapy. Biskind (1943) has cited a similar case of a woman who had 3 spells of compulsive overeating. Each time, she gained about 50 pounds and promptly developed metrorrhagia. On dietary restriction the metrorrhagia disappeared only to recur at the next bout of overeating.

It seems likely that regressions in the growth of mammary carcinomas in animals, observed following administration of yeast extract or yeast extract and riboflavin (Lewisohn *et al.*, 1941, 1942) may have been mediated through enhancement of the estrogen-inactivating function of the liver.

Since, however, the development of a malignant tumor represents a qualitative and, for the most part, irreversible change in the tissue affected, nutritional therapy would in practice be of value mainly in the prevention of pre-neoplastic lesions. As related to those produced by excess estrogen, the maintenance of normal hepatic function appears to be paramount.*

* While this review was in press, Ayre and Bauld (1946) published a report which provides further confirmation of the relation of nutritional deficiency to syndromes caused by failure of inactivation of estrogen in the liver. Thiamine deficiency (as measured by retention of a test dose) was found to occur regularly in patients with menorrhagia and with cancer of the uterus, associated with estrogenic changes in the

III. INFERTILITY

Nutritional deficiency affects the reproductive function in two ways: (1) by a direct effect on cellular metabolism of the reproductive tissues, thus affecting their responsiveness to endocrine principles (*cf.* Hertz and Sebrell, 1944; Hertz, 1945), as well as other functions, and (2) by secondary systemic alterations of endocrine function. Undoubtedly, in a given case, both operate simultaneously and are inseparable. The general literature on this subject has been reviewed by K. E. Mason (1939) and the specific effects of vitamin E in this respect are covered by Mason in Vol. II of *Vitamins and Hormones* (1944).

This discussion is concerned mainly with diminished fertility occasioned by secondary endocrine disturbances and the effects of nutritional therapy. The disturbance which appears to have the most far-reaching effect in this instance appears again to be the rise in active estrogen occasioned by failure of inactivation in the liver. As mentioned on page 153, endometriums obtained from patients during functional uterine bleeding (associated with nutritional deficiency) show a more or less pronounced estrogen effect and little or no secretory effect, suggesting a deficiency or absence of progesterone.

This, of course, may be an etiologic factor in female infertility, and a number of apparent restorations of fertility in such patients have been occasioned by intensive vitamin B complex therapy (M. S. Biskind, unpublished) although the problem has not yet been sufficiently studied. Peters and Footer (1945) have reported that, in patients who bleed excessively owing to the persistence of an acyclic estrogenic endometrium, the endometrium becomes cyclic on vitamin B complex therapy and, in confirmation of the studies described in Section II, the menstrual flow becomes normal.

In the male, not only is spermatogenesis and spermic function affected directly by nutritional deficiency (vitamin A, vitamin E, [*cf.* Mason, 1939], riboflavin [MacLeod, 1942] and undoubtedly other factors) but the rise in body estrogen which results from failure of inactivation in the liver secondarily affects spermatogenesis. Biskind and Falk (1943) studied the effect of therapy with vitamin B complex (in some cases with addition of vitamin E) and found definite increases in sperm counts and in motility, and diminu-

cervical smear. While the method employed by these investigators places emphasis mainly on lack of thiamine (and deficiency of this factor alone would be *sufficient* to impair hepatic estrogen inactivation), it should be remembered that it is virtually impossible for an uncomplicated clinical deficiency of a single factor ever to occur. From the practical standpoint this is extremely important, as administration of thiamine alone is usually of little therapeutic benefit, whereas *complete* therapy (with all the known vitamin B factors together with an adequate natural source) is dramatically effective (see Section VI).

tion in the percentage of abnormal forms, with apparent restoration of fertility in eight of thirteen cases. However, in infertility resulting from vitamin E deficiency (even though addition of vitamin E to the B complex therapy produced striking effects on spermic motility) there was no evidence that this defect was other than irreversible (*cf.* Mason, 1939).

Subsequent experience with vitamin B complex in male infertility has borne out the original experience and Pool (1945) has briefly reported similar favorable results. That the vitamins are not the only nutritional factors of possible clinical importance in spermatogenesis is indicated in a study by Holt *et al.* (1942), on the deleterious effects of deficiency of certain amino acids, especially arginine, on production of spermia in human subjects—a factor of especial importance during wartime restrictions on protein foods. In the treatment of these patients it is important to take into consideration the frequent occurrence of multiple nutritional deficiencies (see Section VI). Nutritional therapy, at least of the type at present available, nevertheless fails to affect fertility in a significant number of patients. These cases also do not respond to direct endocrine therapy of various types—steroids, thyroid, gonadotropins.

IV. THYROID DISTURBANCES AND THYROID THERAPY

The majority of the patients with syndromes related to excess estrogen studied by M. S., G. R. and L. H. Biskind (1944) had a low basal metabolic rate. This was especially true of the patients with signs of severe or moderately severe nutritional deficiency. Administration of thyroid to these patients, in the absence of a vitamin B supplement, usually caused exacerbation of the signs and symptoms of the deficiency without significant change in the metabolic rate. The low metabolic rate in these patients may be the expression of a safety mechanism; the rise in body estrogen resulting from failure of inactivation in the liver depresses the pituitary with diminution in secretion of the thyrotropic principle.

In several patients with enlarged thyroids who were treated nutritionally for syndromes related to excess estrogen (M. S., G. R. and L. H. Biskind, 1944; M. S. Biskind, unpublished), a definite diminution in the size of this gland was observed after several months of therapy with vitamin B complex; this was sufficiently striking to be noticed spontaneously by members of their families. In none of these cases, however, did the goiter completely regress during the period of observation. This is of interest in view of the well-known observation that patients with goiters show periodic further enlargement of the gland during the latter part of the intermenstruum, when the body estrogen rises, and that administration of estrogen to some patients with hyperthyroidism leads to diminution in the basal metabolic rate (Goldman *et al.*, 1940). (Because the administered estrogen causes

further exacerbation of an already tenuous nutritional equilibrium produced by the hyperthyroidism [see Fig. 7] it would seem inadvisable to use estrogen therapy for this purpose unless the nutritional defect were simultaneously corrected.)

Williams and Kendall (1943) have reported that administered thyroid is "less effective in promoting metabolic activity . . . in a state of thiamine deficiency than it is when the intake of thiamine is adequate."

The observations described clarify numerous problems of thyroid therapy that have formerly been both confusing and frustrating. Thyroid is not only one of the most valuable of endocrine substances but it is also one of the most misused. Until the relation of thyroid function (and the physiologic activity of thyroxine) to nutritional status had been clarified, it was impossible to evaluate a number of clinical phenomena related to thyroid function and the metabolic effects of administered thyroid. Among these are: (1) the significance of the basal metabolic rate as an indication for thyroid therapy; (2) the frequent failure of administered thyroid to affect the basal metabolic rate, although side actions such as tachycardia and nervousness may be prominent; (3) the development of endocrine complications (*e.g.*, menorrhagia, cystic mastitis) following the use of thyroid in the treatment of obesity.

It is notable that both the nutritional factors affecting thyroid function operate in the same direction. Failure of inactivation of estrogen in the liver leads to a consistently high blood estrogen which depresses the thyrogenic function of the anterior pituitary, and thiamine deficiency (the effect of other factors has not yet been reported) prevents the development of the normal metabolic effects of thyroxine, whether endogenous or administered. Thus, in the presence of nutritional deficiency, a low basal metabolic rate might be expected and little effect on the metabolic rate would be derived from ingestion of thyroid. Precisely this occurs. In such cases, both the usual assumption that a low basal metabolic rate is a necessary indication for thyroid, and that this can be remedied by giving thyroid, are fallacious. Actually the administration of thyroid in the presence of nutritional deficiency is sharply contraindicated; the sole effect of thyroid therapy in such cases is to cause an exacerbation of the nutritional deficiency, although, probably because of the protective mechanisms discussed, this exacerbation is often not as striking as that produced by estrogen.

In evaluating the basal metabolic rate, it is necessary first to keep in mind the fact that the range of normality is now considered to extend from plus 5 to minus 20% (DuBois and Chambers, 1943) and that both hypothyroidism and hyperthyroidism can occur within this range. Therefore, the clinical status of the patient must be considered in relation to the basal metabolic rate. Often enough, signs usually considered referable to hypo-

thyroidism disappear promptly, solely by correcting the accompanying nutritional defect. The author makes it a practice always first to correct the avitaminosis and, when the lesions are healed, then, and then only, to administer thyroid if this is required, continuing the vitamin therapy at the same time.

Two factors especially have operated to distort the significance of the basal metabolic rate as it is now calculated:—(1) Calculation of the rate to the body surface. (2) Representation of the rate in terms of percentage above and below a theoretically average normal. As Norman Wetzel (1933) has pointed out, heat production is more appropriately considered a function of body mass; in the early work on metabolism, it was related to body surface only as a stratagem to reduce the apparent range of normal variability.

The fallacy of relating the metabolic rate to the body surface is readily seen in cases of extreme obesity. In such cases body weight increases greatly while only a fraction of this increase occurs in body surface. It is common to obtain a basal metabolic rate of plus 10 or plus 20% in a patient with extreme obesity, even when clinical signs point to hypothyroidism. If the oxygen consumption were calculated to the body weight, the metabolic rate would more nearly agree with the clinical condition of the patient.

The practice of calculating the metabolic rate in percentages above and below the "normal" zero serves little purpose but confusion. The fallacious position even of the zero point is indicated by the current shift in the "normal" range proposed by DuBois, already mentioned. Representation of basal metabolic rate in terms of calories per kilogram of body weight per day, even though this provides figures having a wider divergence in the normal range than present practice, would at least give the physician a truer indication of caloric output. In addition this method of calculation would be as useful in obesity as in persons of more nearly average weight.

Thus it is evident that the basal metabolic rate, especially considering the difficulties inherent in the present method of calculation, is not by itself adequate for determining the need for thyroid administration and that estimation of the nutritional status of the patient is of great importance for rational therapy of thyroid disturbances.

Berman (1945) has observed regression in clinical hyperthyroidism under therapy with *p*-aminobenzoic acid (see also the review of Ansbacher, 1944).

V. DIABETES*

The association of deficiencies in accessory dietary factors with certain defects in carbohydrate metabolism has been the subject of numerous

* See also the review of Houssay on the Thyroid and Diabetes in this volume. Ed.

studies. Among substances known to be necessary for utilization of carbohydrate are thiamine, riboflavin, niacin amide, pantothenic acid, ascorbic acid, vitamin A and vitamin D, although the rôle of the latter three is not as well understood as that of the B vitamins (the literature is cited by Rosenberg, 1942). Many investigations have also been made on nutritional deficiency in diabetes and on the effects of nutritional supplements. For the most part, however, these have been concerned with the treatment of specific avitaminotic lesions rather than with any fundamental defect of the disease itself. Duncan (1943), however, has made passing mention of the fact that he had observed an apparent economy of insulin in certain patients on vitamin B complex therapy.

Soskin and his collaborators (Soskin *et al.*, 1934, 1935, 1938, 1939, 1941, 1944), in a series of fundamental investigations, have demonstrated the basic rôle of the liver in maintaining normal carbohydrate balance. They have shown that:—

- (1) In pancreatectomized animals receiving a constant intravenous injection of insulin just sufficient to maintain normal blood sugar, administration of dextrose yields *normal* dextrose tolerance curves.
- (2) In hepatectomized animals with intact pancreas, receiving a constant intravenous injection of dextrose just sufficient to maintain normal blood sugar, administration of additional dextrose yields *diabetic* dextrose tolerance curves.
- (3) When the liver is damaged by a toxic agent, the diabetic type of dextrose tolerance curve is obtained.

C. H. Best (1935) has emphasized the fact that insulin is secreted into the portal circulation. As Waters and Best (1942) have pointed out, "If one were obliged to name the organ in which insulin exerts the most potent influence, there would be little hesitation in selecting the liver."

It has become customary to think of diabetes mainly in terms of insulin deficiency (*cf.* Root and Bailey, 1945). On the basis of an investigation into the nutritional aspects of diabetes, Biskind and Schreier (1945) have suggested an alternative explanation, namely that, owing to impaired function of the liver, the latter organ is no longer able to respond to endogenous insulin, which need not be deficient. Carbohydrate balance could then be restored in two ways, by administration of additional insulin, which (if the functional defect is not too great) forces the recalcitrant liver to behave, or by restoring normal hepatic function so that the liver can respond to pancreatic insulin. Thus the concept of insulin resistance, now restricted to cases in which there is failure of response to exogenous insulin, might be extended to include many more (and perhaps most) cases of diabetes, which they believe are caused by the fact that the liver becomes resistant to the action of endogenous insulin. Depending on the severity

of the functional liver defect, the hepatic response to additional (exogenous) insulin would vary accordingly. Biskind and Schreier believe that impairment of the ability of the liver to maintain carbohydrate balance occurs as a result of nutritional deficiency, and they have shown that intensive nutritional therapy can partly or entirely restore this function.

In a group of 94 diabetics studied by Biskind and Schreier, every one showed signs and symptoms of deficiency of factors of the vitamin B complex. Glossitis occurred in almost all the patients (*cf.* Fig. 12). Cheilosis, nasolabial seborrhea, keratosis of the lower eyelids, splitting of the fingernails in layers, clouding of consciousness, nervousness, insomnia, impairment of memory for recent events, precordial pain or distress, gastrointestinal



FIG. 12 *a*



FIG. 12 *b*

Fig. 12. *a*. Atrophic glossitis in an aged male diabetic, resistant to large doses of insulin, and with severe ketonuria. *b*. After 19 days on intensive oral and parenteral vitamin B complex therapy, during which responsiveness to insulin was restored, and the diabetes could be completely controlled on reduced dosage of insulin; the ketonuria disappeared.

disturbances and polyneuritis were noted frequently. Syndromes related to excess estrogen occurred concomitantly. Among the premenopausal women there was an almost invariable association of menstrual disturbances with the diabetes and the lesions of avitaminosis; among the male patients there was the characteristic lack of libido and potency, usually associated with testicular atrophy. For the most part, all these conditions showed a more or less prompt response to intensive nutritional therapy. Associated with the improvement in the avitaminotic lesions, marked improvement occurred in carbohydrate metabolism. In some cases the insulin requirement could be reduced; in others insulin could be eliminated altogether. Improvement in general health was usually striking.

Fourteen patients required an average daily dose of 41 units of insulin

(range 25 to 80 units) before nutritional therapy and were able to maintain themselves free of glycosuria after vitamin therapy on an average of 18 units (range 10 to 40 units). Sixteen patients previously on insulin required no insulin at all after the nutritional deficiency had been controlled with B complex. Thirty-seven patients, who were all continued for the most part on the same dose of insulin, showed a variety of favorable responses to nutritional therapy:—striking improvement of general health was invariable; there was less tendency to glycosuria; in some there was marked reduction in blood cholesterol levels, improvement in cephalin flocculation tests and other changes indicating improved liver function. Especially striking was the diminished tendency to insulin reactions in patients in whom this had previously been a troublesome factor.

Twenty-five patients who had had diabetes for periods ranging from 3 months to 20 years (average duration 5.9 years), showed striking reductions in fasting blood sugar from an average of 270 mg. per 100 cc. (range 180 mg. to 325 mg.) to an average of 123 mg. (range 100 mg. to 219 mg.) on vitamin therapy alone. Of the series of 94 cases, 2 patients proved refractory to intensive and protracted nutritional therapy, showing no improvement in carbohydrate metabolism and only slight improvement in avitaminotic lesions. Both these patients had very severe indications of avitaminosis B of many years' duration. The failure of response no doubt represents either irreversible tissue changes or failure to supply as yet unrecognized nutritional factors. Significantly, both these patients failed completely to respond to large doses of insulin.

Except in a few cases in which this was not feasible, the factor of diet in the cases reported by Biskind and Schreier was maintained as nearly constant as possible before and during nutritional therapy, until the effect of the latter on carbohydrate balance could be ascertained. Thereafter, whenever possible, the diet was liberalized.

In 1896 Gilbert and Carnot reported that liver had a beneficial but variable effect on diabetes. Following their initial publication numerous French investigators studied the effects of various liver extracts with similar variable results. In 1922 Levine, in this country, reported improvement in 3 of 4 diabetics treated with a special liver extract. And several years later Blotner and Murphy (Murphy and Blotner, 1927; Blotner and Murphy, 1929, 1930) conducted an extremely well-controlled investigation which demonstrated conclusively in patients, that the feeding of raw liver (or of liver fractions other than those containing the antipernicious anemia factor) had a definite effect in lowering the blood sugar of diabetics. Blotner and Murphy, as well as previous workers, thought of this phenomenon as representing the presence in liver of a blood sugar-reducing substance and they even indicated an equivalence of liver by weight with a definite unitage of insulin.

It is, of course, now well known that liver is an excellent source of the B complex and that these factors appear in greatest concentration precisely in the fraction of liver found by Blotner and Murphy to be effective in diabetes; the search for an insulin-like substance in liver was doomed to failure and when De Pencier, Soskin and Best (1934) investigated the effect of liver as a substitute for insulin on nondepleted pancreatectomized dogs, negative results were obtained.

The contrary occurred in other investigations of a similar nature in which depleted animals were used. Martin (1937) observed that the insulin requirement of depancreatized dogs increased steadily during ingestion of a vitamin-deficient diet and that restoration of the vitamins to the diet could restore the responsiveness to insulin provided the deficiency had not progressed too far. Gaebler and Ciszewski (1945) found in 3 of 4 pancreatectomized dogs kept on a maintenance dosage of insulin, that withdrawal of the B vitamins from the diet caused exacerbation of the diabetic state and increased the insulin requirement by 50%. Using as a source of the B vitamins, either yeast (which has also been credited in the past with containing a blood sugar-reducing substance [*cf.* Dubin and Corbitt, 1923], and which has a history of use in diabetes almost as long as that of liver), or a mixture of thiamine, riboflavin, nicotinic acid, inositol, pyridoxine, pantothenic acid and *p*-aminobenzoic acid, they were able to produce prompt amelioration of the diabetic state and to reduce the insulin requirement to its former level. Richter *et al.* (1945) have shown that partially depancreatized rats, given free choice of various nutrients, consumed more protein, more fat, much less carbohydrate and more of the components of the vitamin B complex—thiamine, riboflavin, pantothenic acid, pyridoxine and choline—than normal animals given a similar choice. So long as the depancreatized animals remained on the high protein, high fat, high vitamin diet of their own selection, they showed no symptoms of diabetes; on a stock diet symptoms of diabetes appeared promptly.

Ten years before the experiment just cited, Best (1935) pointed out, "Evidence is accumulating that certain accessory food factors may exert an appreciable effect on the intensity of glycosuria in pancreatic diabetes." But that nutritional deficiency has an etiologic relation to diabetes mellitus was actually shown twenty years before that by Casimir Funk and von Schoenborn (1914). They reported that pigeons kept on a "vitamin-free artificial diet" showed disappearance of glycogen from the liver and marked rise in blood sugar. This phenomenon was interpreted by Collazo (1922) as representing the effect of inanition; however, a later experiment by Funk and Corbitt (1923) showed that a vitamin B extract of yeast could reduce the blood sugar and restore liver glycogen. Eggleton and Gross (1925) subsequently showed that, in vitamin B deficiency in rats, the blood sugar is above normal until terminal deficiency occurs, whereupon

the blood sugar diminishes (at the time when liver glycogen has been depleted). This is analogous to the situation which often occurs in the human diabetic, as Soskin (1944) has pointed out, when the diabetic state apparently improves but at the expense of liver depletion.

When examined in the light of the thesis presented by Biskind and Schreier, that diabetes represents an end result of protracted nutritional deficiency and that impairment of hepatic function which thus results leads to failure of hepatic response to endogenous insulin, many previously puzzling facts about diabetes become clear and take their places in a logical system.

Taub *et al.* (1945), in independent studies, have also implicated dysfunction of the liver in many cases of diabetes and have demonstrated favorable results from nutritional therapy.

VI. ON THE TECHNIC OF NUTRITIONAL THERAPY

For adequate nutritional therapy in the human being there are three requisites: (1) Complete therapy; (2) Intensive dosage by routes assuring adequate utilization; (3) Protracted administration.

Most failures, especially in treatment with the vitamin B complex, result from disregard of one or more of these principles (*cf.* Spies, 1943). The use of single drugs has a strong modern tradition in medicine and, as a consequence, the tendency to administer thiamine or riboflavin or niacinamide alone, in an effort to correct lesions showing a predominant deficiency of one of these factors, is widespread. But clinically, single uncomplicated deficiencies rarely, if ever, occur, and considering the difficulties involved in producing such deficiencies in animals under strict laboratory control, it would be extremely surprising to find a deficiency of a single vitamin in a human being even with the most esoteric dietary habits. The tendency to administer mixtures of the known crystalline B vitamins, while an improvement over the use of single factors, produces clinical effects only slightly better. The addition to these mixtures of adequate quantities of yeast, yeast extracts, rice bran extract, suitable liver extracts or whole liver, produces dramatically superior results. For the purpose of supplying necessary nutritional factors that are as yet unidentified, liver is far superior to yeast or rice bran, the most suitable products being the 80% alcohol-insoluble fraction or whole desiccated liver (in doses supplying the equivalent of at least two or three ounces of liver a day).

The fallacy of administering mixtures of crystalline vitamins alone in nutritional deficiency is illustrated by the experiments of M. S. and G. R. Biskind (1942, 1944) illustrated in Figs. 1 and 2 (p. 152). In these experiments it was possible, by producing deficiency of all the factors of the B complex, to impair the estrogen-inactivating function of rat livers that

appeared perfectly normal histologically. However, by administering a mixture of thiamine, riboflavin, pyridoxine and calcium pantothenate as the sole source of B vitamins, the estrogen-inactivating function could be restored but these rats all developed fatty livers containing focal areas of necrosis. This principle is further illustrated by a recent clinical report of T. and J. Gillman (1945) who studied liver biopsies in infantile pellagrins before and during nutritional therapy. The use of a mixture of thiamine, niacin and ascorbic acid or of riboflavin and niacin in these patients not only failed to effect histologic improvement in the fatty livers (which resembled morphologically the rat livers illustrated in Fig. 2 of this review) but caused actual aggravation of the hepatic lesions. T. and J. Gillman demonstrated that "crude" parenteral antianemic liver extract was only moderately superior to the crystalline vitamins used. However, so compelling is the prevailing view that aqueous extracts of liver represent all the activity of whole liver that these workers turned, for an adequate source of essential nutritional factors, not to desiccated whole liver but to desiccated stomach, which they found to be superior to the parenteral liver extract previously used.

It is unfortunate, from the standpoint of nutritional therapy, that liver extracts concentrated mainly with a view to increasing their antianemic potency, were for a long time virtually the only ones available; and only a small fraction of such extracts currently produced are used for the purpose for which they were originally intended—pernicious anemia. Most of them are employed for treatment of conditions for which they are ill-adapted—mainly nutritional deficiency. This is the more regrettable as the whole liver from which the extracts are derived would, if ingested as food, produce dramatically superior results.

An investigation of the effectiveness of whole liver and of various liver fractions in the treatment of avitaminosis was carried out by M. S. Biskind (1944), with a view to elucidating the factors involved in the refractoriness of certain lesions of avitaminosis B, especially certain types of atrophic glossitis. He found that although the great majority of patients had an excellent response to commercial preparations of the vitamin B complex, and the ameliorative effect persisted indefinitely as long as maintenance therapy was continued, in a few of them the atrophic glossitis showed only a temporary response to the B complex (even though other signs subsided), and this lesion then recurred in a more refractory form. Subsequent intensive administration of aqueous liver extracts (together with the crystalline B factors) orally and parenterally, usually failed to have more than a slight effect on this type of glossitis. Addition to this regimen of the known liposoluble vitamins (A, D, E and K) was equally ineffective. However, the ingestion of cooked whole liver in an amount much less than

that from which the ineffective extracts were derived, caused a rapid and complete healing of the tongue. If the ingestion of whole liver was discontinued, the glossitis recurred in a few days. The lesion could again be healed on resuming this therapy and the tongue could be maintained in the normal state indefinitely as long as liver was ingested at least several times a week. But whole liver alone could not control the associated signs and symptoms of nutritional deficiency; these responded to intensive therapy with the water-soluble B factors.

Among the factors missing from the ineffective liver extracts is biotin, which is bound to the protein in liver. In view of the report by Sydenstricker *et al.* (1942) that atrophic glossitis may occur as a result of biotin deficiency in man, a biotin concentrate was administered parenterally in one patient, in a dose providing 50 γ per day; no perceptible effect occurred.

Accordingly, Biskind further investigated the effect of different liver extracts in maintaining or restoring the estrogen-inactivating function of the liver in rats (utilizing the technic of G. R. Biskind and Mark, 1939), when these extracts were added to a vitamin B complex-free diet. The first preparation was an antianemic extract soluble in 70% alcohol; the second was a nonsaponifiable liposoluble extract originally described by Wiles and Maurer (1939), obtained from the portion of liver remaining after the antianemic fraction is separated.

The nonsaponifiable lipoid extract of liver had a definite, but quite limited, effect in preventing impairment of the estrogen-inactivating function of the liver in animals on a vitamin B complex-free diet and in restoring this function after it had been impaired by vitamin B complex deficiency. The antianemic extract had only a slight effect in restoring the mechanism, although this extract could to a certain extent maintain the estrogen-inactivating function in animals not previously depleted. Another aqueous antianemic liver extract, more highly purified by additional alcohol precipitation, was even less effective.

The lipoid extract could neither restore the body weight of animals depleted in the B complex nor maintain it in nondepleted animals. The antianemic fraction likewise could not restore the body weight in depleted animals but could maintain it (and actually permit a further gain) in rats not previously depleted.

In contrast to the limited effects of either extract alone, *a mixture of the water-soluble and liposoluble fractions*, in proportions representing equal amounts of fresh liver, had a striking effect in restoring the estrogen-inactivating function of the liver and in maintaining it. In addition this mixture caused rapid gains in body weight in animals previously depleted.

This evidence provides experimental confirmation for the existence of factors essential to nutrition in the lipoid fraction of liver and suggests

the advisability of combining these liver factors with those now employed in manufacturing commercial preparations for nutritional therapy. Turner and Miller (1943) have obtained from liver lipoids two substances that stimulate production of white blood cells.

The simplest method of administering a combination of aqueous and lipid fractions of liver is to use the whole desiccated unfractionated liver substance. This, as already indicated, has been found to be extremely effective as a source of accessory nutritional factors in the nutritional therapy of syndromes related to excess estrogen by M. S. Biskind (1944) and of diabetes by Biskind and Schreier (1945). Cooperman *et al.* (1945) have found whole desiccated liver to contain a factor or factors (not present in aqueous liver extracts tested) which are essential to nutrition of the monkey.

Failure of absorption is common in severe deficiencies, as changes take place in the gastro-intestinal tract rather early. Many patients therefore require parenteral therapy. However, as the available *parenteral* liver extracts lack essential nutritional factors, it is not as yet possible to administer complete B complex therapy by the parenteral route. Therefore, mixtures of crystalline B vitamins are thus used along with oral administration of the more nearly complete preparations.

The following is the therapeutic regime employed by Biskind and Schreier (1945) in the nutritional therapy of diabetes: The nutritional factors given orally are usually administered in the following daily amounts, in divided doses after meals: 36 to 45 mg. thiamine, 21 to 36 mg. riboflavin, 12 to 27 mg. calcium pantothenate, 200 mg. niacinamide (occasionally increased to 500 mg.), 3 mg. pyridoxine, 210 mg. choline, 27 to 150 mg. inositol, and 60 to 280 γ *L. casei* factor (folic acid). These vitamins were derived in part from crystalline material and in part from brewers' yeast extract, 80 % alcohol-insoluble liver extract, desiccated whole liver or combinations of these (the inositol and folic acid were derived solely from the natural sources); 300 mg. ascorbic acid was often included.

In addition, in cases in which parenteral therapy was required, from 20 to 60 mg. thiamine, 5 to 10 mg. riboflavin, 50 to 250 mg. niacin amide, 5 to 10 mg. pyridoxine and 5 to 50 mg. calcium pantothenate, was given intramuscularly every day or every other day.

Although many patients respond rapidly and dramatically to therapy with the vitamin B factors, not a few have severe lesions of nutritional deficiency which respond slowly despite intensive therapy (*cf.* Kruse, 1942, 1943). Sometimes rather sudden improvement occurs following protracted intensive therapy, as in some of the cases of diabetes observed by Biskind and Schreier (1945). Perseverance is therefore important. And, as already indicated, the importance of including in the nutritional regime

adequate amounts of accessory B complex factors (preferably in the form of suitable liver fractions or desiccated liver or combinations of these) cannot be too strongly stressed. Few patients respond satisfactorily to mixtures of crystalline B factors alone or to those containing, as sources of accessory factors, a few grains of brewers' yeast.

As in other conditions related to nutritional deficiency, large doses of the B complex factors must be administered indefinitely even after all morphological defects have healed, the minimum maintenance dosage at this stage being at least five to ten times the maintenance amounts for normal persons (*cf.* Martin and Koop, 1942) and often much more.

A clue to the greatly increased vitamin requirement of the patient whose avitaminotic lesions have been healed, as compared with the maintenance requirement of the nondepleted person, may perhaps be found in a study by Bessey and Lowry (1944) on factors influencing the riboflavin content of the rat cornea. These investigators found that the level of riboflavin in the cornea reflects the riboflavin intake but that, once the animal has been depleted, administration of riboflavin fails to increase the flavin content of the cornea to the level originally present, suggesting a qualitative tissue change.

This problem requires extensive further investigation, but the mass of evidence points to the occurrence in human nutritional deficiency of the phenomenon noted by Bessey and Lowry. For the depleted individual to utilize the nutritional factors at all, they must apparently be present in high concentration in the body fluids; and concentrations higher than are necessary to the nondepleted person must be maintained indefinitely. The author has observed numerous cases in which diminution of vitamin intake led to rapid recrudescence of the deficiency lesions even years after the original deficiency was apparently "cured."

ACKNOWLEDGMENTS

The author gratefully acknowledges his indebtedness to the following, whose collaboration or cooperation contributed in no small measure to the successful completion of much of the experimental and clinical work discussed in this review: Special acknowledgment is of course due my collaborators, Dr. G. R. Biskind (Lt. Col., M.C., A.U.S.) of San Francisco, Dr. L. H. Biskind of Cleveland, and Drs. H. C. Falk, Herbert Schreier and M. C. Shelesnyak (Lt. Com., U.S.N.R.) of New York. In the clinical studies on syndromes related to excess estrogen, Dr. George Blinick of New York has contributed especially to the gynecologic aspects, and Drs. Alfred Plaut, Henry Brody (Maj., M.C., A.U.S.) and Elizabeth Strauss of New York and Drs. B. S. Kline and Anna May Young of Cleveland have kindly furnished much of the histologic material. Members of the staffs of numerous pharmaceutical houses have been extremely helpful, among them especially Drs. S. Ansbacher, H. E. Dubin, T. H. Jukes, David Klein, T. G. Klumpp, J. B. Rice, E. Gifford Upjohn, and Messrs. W. R. McHargue and Milford H. Wise. The author's investigations reviewed here

were aided by grants from the Lederle Laboratories, Inc., E. R. Squibb and Sons, the United Hospital Fund of New York, The Upjohn Company, Winthrop Chemical Company, Inc., and Wyeth, Inc.

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