area where drinking-water and soil had an extremely high (though variable) content of fluoride.

The intoxication chiefly affected the skeleton, producing typical radiological features of diagnostic value. The teeth also showed characteristic mottling in many cases.

The paraplegia was caused by compression of the spinal cord produced by excessive deposition of fluorides in the vertebral column and resultant narrowing of the spinal canal. It did not differ significantly from other compression paraplegias.

Significantly high levels of fluoride were found in the blood, urine, and bones of these patients; and there was a characteristic histological pattern of skeletal fluorosis.

We are indebted to Dr. J. B. Sirivastva and to Dr. P. Devi, of the Central Research Institute, Kasauli, for the fluoride estimations. Our thanks are also due to Dr. S. Singh for the help with case-records and the field survey, and to Shri G. S. Sekhon for the photographs.

REFERENCES

Khan, Y. M., Wig, K. L. (1945) *Indian med. Gaz.* **80**, 429.
Lyth, O. (1946) *Lancet*, i, 233.
Largent, E. J., Heyroth, F. F. (1949) *J. industr. Hyg.* **31**, 138.
Møller, P. F., Gudjonsson, S. V. (1932) *Acta. radiol. Stockh.* **13**, 269.
Murray, M. M., Wilson, D. C. (1942) *Lancet*, i, 98.
Pandit, C. G., Raghavachari, T. N. S., Rao, D. S., Krishnamurti, V. (1940a) *Indian J. med. Res.* **28**, 533.
— Rao, D. N. (1940b) *ibid.* **28**, 559.
Pillai, S. C. (1938) *Indian med. Gaz.* **73**, 308.
Roholm, K. (1937) Fluorine Intoxication. London.
Satyanarayanamurthi, G. V., Rao, D. N., Venkateswarlu, P. (1953) *J. Indian med. Ass.* **22**, 396.
Shorie, K. L. C. (1945) cited by Khan and Wig (1945).
Shortt, H. E., McRobert, G. R., Barnard, T. W., Nayar, A. S. N. (1937a) *Indian. J. med. Res.* **25**, 553.
— Pandit, C. G., Raghavachari, T. N. S. (1937b) *Indian med. Gaz.* 396.
Siddiqui, A. H. (1955) *Brit. med. J.* **2**, 1408.
Smith, M. C., Lantz, E. M., Smith, H. V. (1935) *J. Amer. dent. Ass.* **22**, 817.
Spira, L. (1942) *Lancet*, i, 649.
Venkateswarlu, P., Rao, D. N., Rao, R. K. (1952) *Indian J. med. Res.* **40**, 4.
Weatherall, J. A., Weidmann, S. M. (1959) *J. Path. Bact.* **78**, 233.

MUTATION, CANCER, AND AGEING

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IRRADIATION of living things by ionising radiation can result in mutation, cancer, and ageing (Hempelmann and Hoffman 1953, Upton 1957). Probably the most important means by which radiation is believed to produce its biological effects is through dissociation of water with the formation of the chemically active free radicals, HO· and HO_2· (Stein and Weiss 1948). These same free radicals might be expected to be produced during the course of the major energy-gaining reactions employed by living things—the reduction of molecular oxygen to water and the conversion of light energy to chemical energy. Also, since reactions involving free radicals are in general not " clean-cut ", some of these free radicals might be expected to produce side-effects through more or less random reaction with cellular components. The presence of free radicals in living systems has been shown by electron paramagnetic-resonance absorption studies (Commoner et al. 1954 and 1957, Ingram 1958). Further, the concentration of free radicals has been shown to increase with increasing metabolic activity in conformity with the hypothesis that free radicals are involved in biological oxidation-reduction reactions (Michaelis 1951, Waters 1946).

If ageing does involve attack on the organism by free radicals as suggested, an increase in the concentration in the organism of compounds, such as mercaptans, capable of reacting rapidly with free radicals, might decrease the rate of attack on constituents such as desoxyribosenucleic acid (D.N.A.) and thus lead to a decrease in the rate of ageing, and therefore to a prolongation of effective life. Two experiments to investigate this possibility have been carried out.

The Experiments

The first experiment (Harman 1957) employed AKR (male) and C3H (female) mice. These mice were used because their life span is short. Although they die for the most part of lymphatic leukæmia (AKR) or mammary carcinoma (C3H), it was believed that they would be suitable for testing the postulated influence of free radicals on ageing; since, if the hypothesis was correct, the average age at which the neoplastic process appeared should be increased. Mice were obtained shortly after weaning, divided into groups of about 30 (10 per cage), and then fed daily (ad lib.) a powdered diet to which was added either nothing or else the hydrochloride of an antioxidant. The mice were weighed and counted every month.

AKR mice receiving cysteine hydrochloride (1·0% w), 2-mercaptoethylamine hydrochloride (1·0% w), or 2, 2′-diaminodiethyl disulphide dihydrochloride (0·5% w) had a half-survival time (age by which 50% of the animals were dead) of 10·5, 10·5, and 10·6 months respectively, whereas that of the controls was 7·6 months—a prolongation of about 35% ($P < 0·01$). The influence of these compounds is not on the leukæmia which the AKRs develop, as shown by the fact that all but one of the blood-smears taken at 12 and 13 months were normal. The mouse from which the abnormal smear was taken was moribund. Further, the weight gains of the control and treated mice were essentially the same. Ascorbic acid (2% w) and 2-mercaptoethanol (0·5% w) were also studied; neither prolonged life. None of the five compounds tested had any definite effect in the C3H mice.

The second experiment (Harman 1960b) utilised Swiss mice (males) in addition to the AKR (males) and C3H (female) strains. Pelleted food—to which either nothing or the hydrochloride of one of four antioxidants was added before pelleting—was used instead of powdered food in hope of maintaining high tissue levels of the additives for longer periods. Fresh batches of food were made up at intervals of 1 to 3 months.

Cysteine hydrochloride (1% w) and hydroxylamine hydrochloride (2% w) increased the half-survival time of the AKR mice from the control figure of 9·6 months to 11·0 and 11·2 months respectively—a prolongation of about 15%. 2-Mercaptoethylamine hydrochloride (1% w) did not increase the life span—for some reason the members of this group of AKR mice had a relatively high mortality in the early months of the experiment. 2, 2′-diaminodiethyl disulphide was apparently toxic at the level employed (1% w) for this as well as the other two strains of mice.

2-Mercaptoethylamine hydrochloride (1% w) increased the half-survival time of C3H mice from a control value of 14·5 months to 18·3 months—an increase of 26% ($P < 0·01$). There was some indication (decreased mortality-rate) during the early months of the first experiment that 2-mercaptoethylamine might lengthen the life span; the pronounced effect in the second experiment may be in part due to the difference in the mode of feeding. Hydroxylamine hydrochloride (1% w) slightly prolonged the half-survival time from 14·5 months to 15·5 months—an increase of about 7%. In this, as in the first experiment, cysteine hydrochloride (1% w) did not prolong life.

None of the antioxidants studied lengthened the life span of the Swiss mice.

Discussion

These two experiments are encouraging and justify further exploratory studies of antioxidants as anti-ageing agents; several additional antioxidants, shown to protect mice against multiple sublethal doses of X-radiation

(Ershoff and Stern 1960), are now under study in the AKR, C3H, and Swiss mice. In addition, the magnitude of the life-prolonging effect, particularly that of 2-mercaptoethylamine hydrochloride, suggests that endogenously produced free radicals play a major part in ageing.

These results agree with those of a human study (Chope and Preslow 1956) which indicates that older persons with low blood-levels of vitamin A and ascorbic acid (both easily oxidised compounds) have a higher mortality-rate than do persons with greater amounts of these substances in the blood.

The concept that free radicals are involved in ageing was applied to the fact that the rate of ageing as measured by the logarithm of the mortality-rate increases with advancing age. Part of this effect could be due to a decrease in the level of antioxidants—for example, mercaptans—in the body with increasing age. A study of the serum-mercaptan concentration as a function of age agreed with this possibility (Harman 1960a). The serum concentration of mercaptan groups of normal men decreased with age from a level of about 55 μmoles per 100 ml. of serum at age 20–40 to about 40 μmoles per 100 ml. at 80. The data for women are of the same magnitude and show the same trend. The serum concentration of another antioxidant, ascorbic acid, likewise has been reported to decrease with age in men (Kirk 1954).

Thus, on both theoretical and experimental grounds, there is firm support for the hypothesis that endogenously produced free radicals play a prominent part in mutation, cancer, and ageing.

Summary

Mutation, cancer, and ageing are attributed basically to the side-effects of endogenously formed free radicals.

Experimental work on ageing based on this hypothesis has been encouraging. Several antioxidants were found to increase the half-survival time of AKR and C3H mice; the prolongation with 2-mercaptoethylamine was about 20%. Serum-mercaptan levels, as predicted by the free-radical hypothesis, were found to decrease with age.

REFERENCES

Chope, H. D., Breslow, L. (1956) *Amer. J. publ. Hlth*, **46**, 61.
Commoner, B., Townsend, J., Pake, G. E. (1954) *Nature, Lond.* **174**, 689.
— Heise, J. J., Lippincot, B. B., Norberg, R. E., Passonneau, J. V., Townsend, J. (1957) *Science*, **126**, 57.
Ershoff, B. H., Stern, C. W., Jr. (1960) *Proc. Soc. exp. Biol.* **104**, 274.
Harman, D. (1957) *J. Gerontol.* **12**, 257.
— (1960a) *ibid.* **15**, 38.
— (1960b) *ibid.* (in the press).
Hempelmann, L. H., Hoffman, J. G. (1953) *Annu. Rev. nuclear Sci.* **3**, 369.
Ingram, D. J. E. (1958) Free Radicals as Studied by Electron Spin Resonance. New York.
Kirk, J. E. (1954) *Nutr. Symp. Ser.* **9**, 73.
Michaelis, L. (1951) *in* The Enzymes (edited by J. B. Summer and K. Myrback); vol. II, chap. 44. New York.
Stein, G., Weiss, J. (1948) *Nature, Lond.* **174**, 650.
Upton, A. C. (1957) *J. Gerontol.* **12**, 306.
Waters, W. A. (1946) The Chemistry of Free Radicals. London.

Preliminary Communication

LUCANTHONE RESINATES IN SCHISTOSOMIASIS

LUCANTHONE hydrochloride (' Miracil D ') was introduced [1] for oral treatment of schistosomiasis. It is 1-β-diethylaminoethylamino-4-methylthioxanthone hydrochloride. It has been successfully used for twelve years, in which its main drawback has always been the severity of gastrointestinal side-effects. There have been several attempts to present lucanthone in a less irritant form,[2 3] but the hydrochloride remains in general use.

The description by Abrahams and Linnell [4] of oral depot therapy with drug resinates suggested that lucanthone resinates might produce adequate blood-levels without the unpleasant side-effects associated with the hydrochloride. Prof. W. H. Linnell kindly arranged for two different lucanthone resinates to be supplied for laboratory trials. The lucanthone was combined with ' Zeocarb 225 H ' resin of 1% and 8% cross-linkage. The plasma levels after single doses equivalent to 1 g. of lucanthone base were estimated by an acetone-ether extraction method [5] in baboons and in man. Satisfactory blood-levels were obtained with the 1% cross-linkage compound but not with the 8% compound, and side-effects were minimal.

When the same resinates were given, in doses of 1 g. lucanthone equivalent twice daily for three days, to patients in East Africa, the 1% cross-linkage resinate maintained a curative blood-level of over 0·5 mg. per 100 ml.[6] but the 8% resinate did not. Cures were obtained with the 1% resinate, and side-effects were not troublesome. When further supplies of lucanthone resinates were available, trials were begun on East African patients in Kenya.

1. Kikuth, W., Gonnert, R. *Ann. trop. Med. Parasit.* 1948, **42**, 256.
2. Deschiens, R. *Bull. Soc. Path. exot.* 1951, **44**, 667.
3. Newsome, J. *Trans. R. Soc. trop. Med. Hyg.* 1951, **44**, 611.
4. Abrahams, A., Linnell, W. H. *Lancet*, 1957, ii, 1317.
5. Newsome, J., Robinson, D. L. H. *Trans. R. Soc. trop. Med. Hyg.* 1960, **54**, 454.
6. Halawani, A., Hafez, A., Newsome, J., Cowper, S. G. *J. R. Egypt. med. Ass.* 1949, **32**, 29.

METHODS

Two compounds were used—a 1% cross-linkage resin containing 36·4% lucanthone, and a 2% cross-linkage resin containing 41·3% lucanthone. The dose of the 1% resinate was dispensed in three cachets and provided the equivalent of 1 g. of lucanthone. The 2% resinate was given in doses of three cachets containing a total of 0·9 g. of lucanthone. All doses were given under personal supervision.

COMPARISON OF SIDE-EFFECTS DURING ONE COURSE OF TREATMENT WITH HYDROCHLORIDE AND RESINATE OF LUCANTHONE IN AFRICAN ADULTS

Side-effects	6 g. hydrochloride: no. affected among 11 patients	12·1 g. resinate 2% cross-linkage*: no. affected among 24 patients
Nil	1†	16
Vomiting	7	2
Nausea	10	3
Anorexia	8	2
Epigastric discomfort or pain	6	6
Vertigo	6	0
Depression	2	0
Prostrate	3	0
Toxic psychosis	1	0

*=5·4 g. of base. † Incomplete course.

Adult patients infected with *Schistosoma hæmatobium* and *S. mansoni* were chosen for their high output of viable eggs and their normal renal function. After treatment, urine or fæces were examined on each of three consecutive days monthly for two or three months by microscopy, a hatching technique, and rectal biopsy.[7] Plasma levels were estimated, in most cases, at intervals of one or two days.

RESULTS

Lucanthone Resinate 1% Cross-linkage

Eight patients were treated by the lucanthone equivalent of 1 g. twice daily for three days, and the results were compared with those in eight patients treated with lucanthone hydrochloride on the same dosage schedule.

Plasma levels from patients receiving resinate were in general satisfactory, but showed greater individual varia-

7. Davis, A. *E. Afr. med. J.* (in the press).