CHAPTER 6

TOXIC EFFECTS OF LIVER EXTRACTS ON MICE

MOTIVE AND TECHNIQUE OF THE EXPERIMENTS

In order to obtain a measure of the toxic effect of large doses of liver extract the author tried giving 1 cc. liver extract subcutaneously to mice. If this injection killed the mouse, and effort was made to determine the minimum lethal dose by diluting the extract. When an extract was found to be lethal for mice, it was treated in various ways in order to remove the toxic factor; by this way it was also possible to determine some of the properties of this factor. Nothing could be found in the literature on the subject.

A total of 225 mouse experiments were performed in Gea Ltd's experimental laboratory. The time of the occurrence of the symptoms after the injection, and the toxic symptoms themselves, are not recorded, mortality being the deciding factor. Some of the mice had convulsions, others lay motionless, and others again were "jerk mice" (which means that the slightest exterior contact produced violent reactions). The type of reaction depended on the extract employed.

EXPERIMENTAL RESULTS

The results of the mouse experiments are given in four tables. One shows the toxic effects of the commercial extracts, the other three the effects of the experimental extracts.

Effects of commercial extracts: Table 13 (p. 69)

It will be seen from the table that the toxic effect on mice decreases with the content of dry substance. (Campolon → Gea BF_1).

TABLE 13

Toxic effect of liver extracts on mice

Commercial extracts

Liver extracts employed	Dry subst. in g. per 100 g. extr.	Dilution of extract	No. of mice used	Affected but survived	Died	Aggregate result
Campolon	27.3	Undiluted	5	2	3	
		1—1	5	2	3	+
		1—25	5	5		
Hepsol fort.	24.2	Undiluted	5		5	++
		1—1	5	3	2	+
Lilly's extract		Undiluted	5		5	++
Exhepa fort.	2.9	„	5	5		(+)
Gea A	0.9	„	15	6		(+)
Gea BF_1	0.6	„	10	0		—

Some mice affected, but none died = (+)
Some mice affected and some died = +
All mice died = ++

Whereas Gea A has only little effect, Gea BF_1 has no effect, and consequently the butyl-alcohol was able to remove the mouse-toxic factor.

A comparison between the toxic effect on mice and the unpleasant reactions in man will show that if a preparation is inclined to cause primary symptoms in man, it is also toxic to mice; on the other hand these mouse experiments cannot say anything definite as to an extract's tendency to induce secondary unpleasant reactions.

Effects of experimental extracts: Tables 14a (p. 70), 14b (p. 71) and 15 (p. 72).

The result of the tests with Gea iron-precipitate 1 will be found in Table 14a (p. 70). The extract is highly toxic, which agrees well with its high percentage of dry substance. Working on the basis of Dodds'[40] report on the reducing and prolonging effect of zinc-acetate on the biological reactions of histamine, the author tried injecting 0.1 cc. zinc-acetate before injecting the liver extract, and also tried adding

TABLE 14a

Toxic effects of liver extracts on mice

Experimental extracts

Liver extract employed	Dry subst. in g. per 100 g. extract	Dilution, or other treatment of extract	No. of mice used	Affected but survived	Died	Aggregate result
Gea iron precipitate 1	16.9	Undiluted	5		5	++
		+ previously inj. 0.1 cc. zinc-acetate	5		5	++
		Mixed with 1 % zinc-acetate	5		5	++
		Mixed with 1.5 % zinc-acetate	5		5	++
		Treated with pancreas-erepsine (4) or with intest.-erepsine (4)	8		8	++
		Precipitated with phosph.-tungstic acid	5		5	++
		Treated with permutite	10	2	8	+
		Treated with aluminium gel	5	5		(+)
		Boiled 5 hrs	5		5	++
		Dissolv. in 80 % alcohol, prec. with 3 vols. acetone	Filtrate 5		5	++
			Precipitate 5			−

Regarding the signs (+), +, ++ see Table 13 (p. 69)

TABLE 14b

Toxic effects of liver extracts on mice

Experimental extracts

Liver extract employed	Dry subst. in g. per 100 g. extract	Dilution, or other treatment of extract	No. of mice used	Affected but survived	Died	Aggregate result
Gea B precip. with acetone	0.8	Filtrate	1		1	++
		Precipitate	1			−
Gea iron precip. 2	0.5	Undiluted	10	1	9	++
		Diluted 1—1	10	1	9	++
		Diluted 1—2	5	4	1	+
		Diluted 1—3	10	7	2	+
		Diluted 1—5	5	2		(+)
		Precipitated with 80 % alcohol	5		5	++
		Treated with zinc-acetate	5		5	++
		Treated with erepsin, then added acetone	10	2	8	+
Gea butyl-residue			10			−

Regarding the signs (+), +, ++ see Table 13 (p. 69).

1 per cent., later 1.5 per cent. zinc-acetate to the liver extracts prior to injection; this did not alter the toxic effect, however. Furthermore, Professor Ege kindly treated Gea iron-precipitate 1 with permutite in order to remove the histamine, but the extract was still toxic, though slightly less so than the untreated extract.

As was mentioned above, Isaacs[83] employed permutite for the removal of histamine from liver extracts.

Histamine therefore does not seem to be the cause of the death of

TABLE 15

Toxic effects of liver extracts on mice

Experimental extracts

Liver extract employed	Dilution, or other treatment of extract	No. of mice used	Affected, but survived	Died	Aggregate result
Pernæmi "Nyco" product No. 18	Undiluted	5		5	++
	Diluted 1—9	5			–
	Mixed with 2 % zinc acetate	5		5	++
	Standing with torantil with and without buffer	10		10	++
	Digested with pancreas-erepsine (5) or with intest.-erepsine (5)	10		10	++
	Treated with aluminium gel	5			–

Regarding the signs (+), +, ++ see Table 13 (p. 69)

the mice; what is more, mice are relatively little sensitive to histamine. As the table shows, Gea iron-precipitate 1 was also treated with phosphor-tungstenic acid to precipitate the adenosine, but this made no change in the toxic effect of the extract. Nor can the toxic factor be a simple polypeptide, as the factor is not destroyed by erepsine digestion. Boiling Gea iron-precipitate 1 did not destroy the mouse-toxic factor; on the other hand, the precipitate from the alcohol-acetone precipitation caused no reaction in mice, whereas the filtrate had the usual powerful effect. The second method of removing the toxic factor was treating with an aluminium gel, but the question of whether the anti-pernicious principle was also removed or not by this method was not examined.

Gea iron-precipitate 2, which differs from No. 1 merely in that pH is 5 instead of 4 when precipitating the protein, is surprisingly

toxic when its low dry-substance content is considered (4.6 mg. dry substance per injection), and it is lethal even in a dilution of 1—3 (Table 14b, p. 71). The fact that the lethal dose can be titrated shows that the quantity of toxic factor is the vital point and that there is no question of allergic reaction. Erepsine treatment and the addition of zinc-acetate gave the same result for Gea iron-precipitate 2 as for Gea iron-precipitate 1; furthermore, precipitation with 80 per cent. alcohol was also tried, but the toxic factor was no more destroyed or removed by this process than by the others.

Table 15 (p. 72) merely confirms the other two tables and gives the results of some additional tests. The extract employed was Pernaemi, a Norwegian commercial extract in which 1 cc. = 10 g. liver. It proved to be very toxic to mice; zinc-acetate and erepsine digestion had no effect on the toxic factor, which on the contrary was removed with aluminium gel. Acting on Felix's[19] directions the author allowed Pernaemi to stand with Torantil under the optimal conditions in order to dehistaminise the preparation, but the toxic effect was not altered.

CONCLUSION

The experiments show that mice can be used to decide whether a commercial extract is primarily toxic or not. But mouse experiments cannot decide whether an extract may or may not cause secondary unpleasant reactions.

It is shown that the toxic factor for mice cannot be histamine (permutite tests — zinc-acetate tests — torantil tests) or adenosine (phosphor-tungstenic acid tests), or a simple polypeptide (erepsine tests); that the factor is soluble in 80 per cent. alcohol (Gea B), and that it is not precipitated from this solution by adding 3 volume parts of acetone (Gea A treated ad modum Best et al.[12] filtrate and precipitate); and finally, that the factor is separable from the anti-pernicious principle by shaking with butyl-alcohol, whereby the toxic factor passes into the butyl-alcohol where it disappears (to great a dilution?) The toxic factor can kill mice in quantities as small as 1.5 mg. (the dry substance in Gea iron-precipitate 2, dilution 1—3), and the toxic factor is not identical with the anti-pernicious principle (Gea BF_1: + anti-pernicious principle but not toxic to mice).

Consequently, the mouse-toxic factor has the same characteristics as the factor which induces the primary unpleasant reactions in man, so that the two factors may possibly be identical.