LETTERS TO THE EDITOR

The Dietary Toxicity of Glycerol Formal in the Rat

SIR,—As an extension to recent studies on the acute toxicity of glycerol formal before its adoption as a solvent for use in toxicity testing[1], a short test on the dietary toxicity to the male rat has now been completed.

Groups of ten male rats of Wistar strain, of average weight 90 g., were maintained for sixteen weeks on a diet of an aqueous paste of MRC diet 41B meal to which respectively 0, 316, 1000, 3162 and 10,000 p.p.m. by weight of glycerol formal had been added. In addition to observations for death and toxic effects, daily food consumptions and weekly body weight changes were measured. During this period, the control, 316 and 1000 p.p.m. groups showed no significant differences of any kind. The 3162 p.p.m. group showed a slight but significant reduced rate of weight gain (90–94 per cent of that of the controls), but no other apparent effects, though there was one death in this group in the fourteenth week, probably due to a kidney infection. The 10,000 p.p.m. group showed a reduction of rate of weight gain, falling to 70–80 per cent of the controls after 4 weeks. The animals were obviously weak and undernourished, but showed no decreased food consumption or specific toxic effects. Six rats in this group died between the fifth and the thirteenth weeks, apparently due to gastrointestinal infections possibly induced by lowered resistance. The survivors of this group were killed after thirteen weeks, and showed no specific macroscopic pathology other than that associated with undernourishment; relative liver and kidney weights were higher than normal, probably due to malnutrition.

After sixteen weeks, it was decided to examine the remaining groups for derangement of drug-metabolising functions (e.g., liver-enzyme balance). This was done by adding to the diets of all groups the same quantities of the organophosphorus insecticide rogor (*OO*-dimethyl-*N*-methylcarbamoylmethylphosphorodithioate), whose *in vivo* anticholinesterase properties are dependent on the balance of a number of activating and detoxifying enzyme systems, mainly in the liver. It was thought that similar rates of cholinesterase inhibition between groups when this substance was administered would be a good index of metabolic normality. Accordingly, while the glycerol formal treatment continued, the diets of all groups were further supplemented by 25 p.p.m. of pure rogor for four weeks, and then 50 p.p.m. for three weeks, while weekly heart blood samples were taken for plasma and erythrocyte cholinesterase activity determinations by a standard manometric method[2]. During this period, the previous pattern of weight gain and absence of toxic effects continued, and there was no difference between cholinesterase activities of the groups, except for a marginally greater degree of erythrocyte cholinesterase inhibition in the 3162 p.p.m. group.

All animals were then killed, after a total of 23 weeks, and showed no macroscopic pathology or significant group variation of liver or kidney weights, or of brain cholinesterase and no liver or kidney histopathology at 1000 p.p.m.

Hence it may be concluded that incorporation of up to 1000 p.p.m. of glycerol formal in the diet of male rats (equivalent to 75–150 mg./kg./day, according to body weight), caused no significant toxic effects or metabolic changes. This amount is therefore considered satisfactory for use as solvent in dietary toxicity testing of solid materials insoluble in other less toxic solvents; inclusion of a

solvent control is advised. Higher amounts caused reduced weight gain, disturbance of drug metabolism, and increased susceptibility to infection.

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May 27, 1959.

REFERENCES

1. Sanderson, *J. Pharm. Pharmacol.*, 1959, **11**, 150.
2. Fenwick, Barron and Watson, *Biochem. J.*, 1957, **65**, 58.

Mast Cell Response in Aseptic Inflammation

SIR,—It has been reported previously that when rats are infected with *Staphylococcus aureus*, there is widespread degranulation of tissue mast cells.[1] The histamine content of the skin is increased after remote surgical injury,[2] and as much of the histamine in the skin is contained in the mast cells,[3] it was of interest to study the mast cell changes in the subcutaneous tissue of the rat during aseptic inflammation.

Groups of 6 rats of approximately 150 g. body weight, of either sex, were employed for each observation. Four small cotton wool pellets soaked in spirit of turpentine were implanted under the dorsal skin under ether anaesthesia. Groups of animals were killed at intervals and the resulting changes were studied.

There were no mast cells in the inflamed area, and degranulation and swelling of the cells was seen in the adjacent areas after 6 hours. The maximum changes were noted 24 hours after injury, when almost all the cells in the adjacent areas were ruptured, and metachromatic materials were seen lying free in the tissue. After a further 24 hours, free metachromatic material had disappeared, presumably having been washed out, or phagocytosed[4] by the inflammatory exudate. The inflammatory response was also very much reduced at this time. At the end of 72 hours there was considerable proliferation of new mast cells along side the blood vessels in the adjacent areas. In the next 24 hours, the cells were seen to have migrated away from the blood vessels towards the inflamed area and form cell clumps. In many cell clumps the outline of the individual cells could not be distinguished and it is possible that this was the result of rapid cell division without complete separation of the cytoplasm. The cells were also partly degranulated. By the end of 7 days the cells, though still partly degranulated had separated. At this time there was development of fibrous tissue round the implanted pellets. The fibrosis was very much marked in 10–14 days, when the mast cells presented a nearly normal appearance.

After intraperitoneal injections of turpentine, it has been previously noted[4] that there is rupture of mast cells, inflammation and necrosis, but not much of cell proliferation. The disappearance of mast cells in the inflamed area, may possibly be due to the effects of turpentine, but the changes noted in the adjacent areas are most likely to be related to the inflammatory changes. The earlier disruptions of mast cells may cause liberation of histamine, and thereby increase vasodilatation and capillary permeability, and thus help in forming the inflammatory exudate. The later proliferation of mast cells and invasion of the

inflamed area is followed by development of fibrous tissue. In view of the fact that mast cells may secrete hyaluronic acid,[5] it is possible that these two changes may be related. A full report of these findings will appear elsewhere.

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May 30, 1959.

REFERENCES

1. Mishra and Sanyal, *J. Pharm. Pharmacol.*, 1959, **11**, 127.
2. Geiringer and Hardwick, *J. Physiol.*, 1953, **119**, 410.
3. Riley and West, *A.M.A. Arch. of Derm.*, 1956, **74**, 471.
4. Gustafsson and Cronberg, *Acta Rheum. scand.*, 1957, **3**, 189.
5. Asboe-Hansen, *Ann. rheum. Dis.*, 1950, **9**, 149.

The Influence of Chlorpromazine on Vascular Effects of Vasopressin and some other Pressor Agents in Dogs

SIR,—Chlorpromazine has been found to potentiate the antidiuretic action of endogenous as well as exogenous vasopressin in rats[1,2]. It is assumed that this potentiation might be due to the inhibition of the enzymes which normally break down vasopressin in the body. If it were so, the other effects of vasopressin should be potentiated by chlorpromazine too. Therefore the vascular effects of vasopressin after chlorpromazine were studied. Some other pressor substances were also investigated.

Normotensive dogs were anaesthetised with a mixture of chloralose (70 mg./kg.) and urethane (1 g./kg.). Carotid blood pressure was recorded by means of a mercury manometer. All injections were made by the intravenous route. A submaximal dose of the pressor agents was given before and after chlorpromazine.

Chlorpromazine in doses of 1-5 mg./kg. markedly augments the vascular effects of vasopressin (Tonephin: Hoechst), angiotonin and ergotamine. This potentiating effect manifests itself about 10 minutes after the injection of chlorpromazine and persists for several hours. Barium chloride as a nonspecific pressor agent was used as control in all experiments. It was found that pressor effects of barium chloride are not augmented by chlorpromazine. The same holds true for 5-hydroxytryptomine and lysergic acid diethylamide. According to Kopera[3] neither the vascular effects of sympathomimetic drugs and central nervous system stimulants are potentiated by chlorpromazine. It seems therefore that chlorpromazine potentiates only those pressor agents which contain peptide linkages. Since the pressor peptides used in this study are rather chemically different substances, the chlorpromazine induced potentiation is likely to be ascribed to the inhibition of a nonspecific peptidase.

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REFERENCES

1. Kovacs, Kovacs, Kovacs and Petri, *Arch. int. pharmacodyn*, 1957, **109**, 1.
2. Supek, Kečkeš and Vojvodić, *Arch. int. pharmacodyn.* 1959, in the press.
3. Kopera, Wiener klin. Wschr. 1955, **67**, 867