The Effect of N'-Nitro-N-Nitroso-N-Methylguanidine on the Liver After Administration to the Rat

Nitrosoguanidine (NG) is very widely used as a mutagen for bacteria[1] and for other microorganisms[2], and has been shown to produce stomach tumours after administration to the rat[3], to produce fibrosarcomas on s.c. injection[4], and to cause tumours of the glandular stomach when administered in the drinking water[5]. SUGIMURA and FUJIMURA[5] mention that, apart from lesions in the intestine, other organs were almost unaffected, except for 1 case of liver adenoma. The present communication describes a very spectacular change in liver, in which almost the entire organ was converted into cysts. Similar changes have been observed by Dr. R. SCHOENTAL (unpublished communication).

The compound was administered as a suspension (20 mg in 2 ml water) by stomach tube. Six 200 g female rats of a Porton derived strain were used, the animals receiving either 1 dose, 2 doses with a 2-h interval, 2 doses with a 1-h interval, 3 doses at 2-h intervals, 3 doses at 1-h intervals, or 4 doses at 1-h intervals. The animals were maintained on MRC diet 41B, and either died or were killed approximately 2 years later. In each case, the major part of the liver was found to be converted into large cysts filled with a yellowish gelatinous material (Figure 1). The lobe on the left, which in the photograph appears dark, is in fact a mass of cysts. On microscopic examination, the cysts were found to be simple biliary cystadenomas (Figure 2). In the remaining liver, there was no evidence of cirrhosis or of regenerative nodules. The hepatic parenchymal cells showed considerable variation in nuclear size, some nuclei being hyperchromatic and enlarged. This appearance is similar to that described following administration of hepatic carcinogens[6,7]. Each animal had squamous cell carcinomas of the stomach. 1 animal had fibrosarcoma of the leg which had metastasized to the lung, and another had an adenocarcinoma in the porta hepatis, derived either from bile duct or pancreatic duct.

A problem therefore arises in that there exist a number of biologically very potent compounds which are all known to be capable of reacting as alkylating agents with various cell constituents, so that it might be plausible to suggest that an alkylation reaction of some sort is responsible for the biological effects observed, and yet the compounds have rather different actions in the intact animal. For example, NG is known to methylate DNA[8-10], and it causes the formation of liver cysts, while no kidney tumours were detected. Nitrosomethylurea also methylates nucleic acids and produces tumours in many tissues, but none have been detected so far in liver[12]. Dimethylnitrosamine methylates nucleic acids in liver and kidney in the intact animal[11], and a single injection gives rise to kidney tumours, but it has no apparent long-term effect on liver after injection into the adult animal. It is possible that the differences in the biological effects of these com-

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Fig. 1. Liver of rat given 2 doses of NG, each of 20 mg, with an interval of 1 h, and killed after 2 years 1 month. With the exception of the small caudate lobe, the tissue has been converted almost entirely into a spongy mass of cysts.

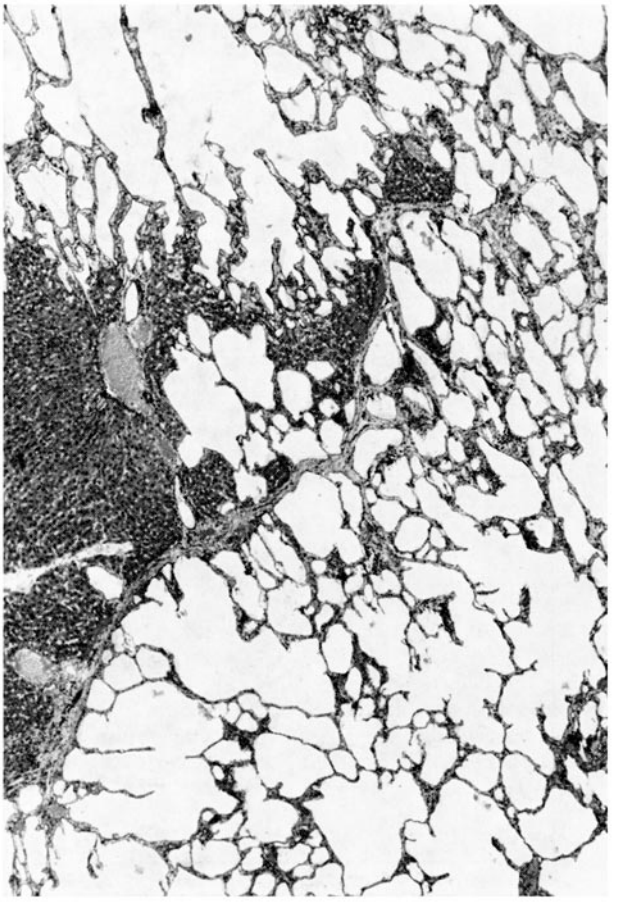

Fig. 2. Biliary cystadenoma found in female rat given 2 doses of NG, each of 20 mg, with an interval of 1 h, and killed after 2 years 1 month. Haematoxyline-eosin staining. × 50.

pounds are due to such factors as the half-lives of the compounds and their distribution in the animal, or there may be fundamental differences in the nature of the reactions with nucleic acids, proteins, or other cell constituents, or, of course, alkylation may not be the relevant reaction for biological activity. As compounds of this type are used in cancer chemotherapy, as well as in the study of carcinogenesis and mutagenesis, individual differences in the biological activities of different alkylating agents merit further investigation[12,13].

Résumé. La nitrosoguanidine administrée à des rats par sonde stomacale provoque la formation de très nombreux kystes biliaires dans le foie et, dans chaque cas, de tumeurs d'estomac squameuses.

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[13] I would like to thank Dr. W. H. Butler for evaluation of the histology, and R. Hunt and C. R. Kennedy for technical assistance.

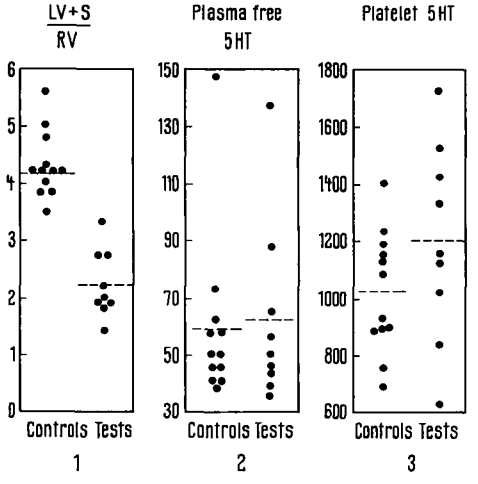
Blood 5-Hydroxytryptamine in Rats with Pulmonary Hypertension Produced by Ingestion of *Crotalaria spectabilis* Seeds

The prolonged oral administration of *Crotalaria spectabilis* seeds to rats induces pulmonary hypertension[1]. This is associated with cardiac enlargement due to right ventricular hypertrophy[2,3], thickening of the pulmonary trunk[4] and medial hypertrophy and arteritis in the muscular pulmonary arteries[3,5]. The active principle of *C. spectabilis* seeds is the pyrrolizidine alkaloid monocrotaline[6] and administration of the pure alkaloid induces pathological changes which are identical to those caused by the seeds[7].

The mechanism by which monocrotaline induces pulmonary hypertension is unknown. However, in this connection Takeoka, Angevine and Lalich[8] reported that proliferation of mast cells occurred in the lungs of rats fed on *C. spectabilis* seeds. Rat mast cells are rich in 5-hydroxytryptamine (5HT)[9] which is known to be a pulmonary vasoconstrictor in many animal species[10] and its release from mast cells may play a part in the normal regulation of vascular tone[11]. It was considered possible that the pulmonary hypertension induced by monocrotaline might be associated with abnormal 5HT release. Accordingly, experiments were conducted in which the plasma-free and platelet-bound 5HT levels were measured in rats fed on *C. spectabilis* seeds and the results compared with those obtained in rats given a normal diet.

Methods. Twenty-one female Wistar albino rats (initial weight 68–87 g) were divided into 2 groups consisting of 9 test animals and 12 controls. The test rats were given a diet of powdered Thomson rat cubes to which finely ground *C. spectabilis* seeds had been added to give a concentration of 1 g/kg diet. The control rats received a diet of unadulterated powdered rat cubes. On the thirty-third day of the experiment the rats were anaesthetized with ether and blood was withdrawn by cardiac puncture for 5HT assay. Determinations of plasma-free and platelet-bound 5HT were made using a spectrophotofluorimetric method[12,13]. After withdrawal of blood the animals were killed and their thoracic organs immersed in formalin. When fixation was complete the hearts were dissected free and their chambers were weighed separately using a method described previously[3]. The right ventricular weight was expressed as a ratio of the weight of the left ventricle and interventricular septum for assessment of right ventricular hypertrophy, the presence of which was accepted as evidence of pre-existing pulmonary hypertension.

Results. In the 12 control rats the ratio obtained by dividing the right ventricular weight (RV) into the weight of the left ventricle (LV) and interventricular septum (S) ranged from 3.5–5.6 with a mean of 4.2. The range in the 9 test rats was from 1.4–3.3 with a mean value of 2.2. The difference between these 2 means is highly significant ($P < 0.001$), indicating that all the test rats had right ventricular hypertrophy when compared with the controls (Figure 1).



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