an additional lightly marked furrow toward its proximal boundary.

The 11 head regenerates considered, for the present, to be abnormal fall into two classes: those which are asymmetrical, perhaps because of some environmental factor such as temperature and those which have additional furrows symmetrically demarcated ventrally, or ventrally and laterally. Those in the second group and the condition indicated in segment ii (3) could be interpreted as indicating the possibility that additional segments may be added to the head regenerate later.

References

1. HYMAN, L. H. *Amer. Nat.*, 1940, **74**, 513–527.
2. MICHEL, A. *Bull. Sci. France et Belgique*, 1898, **31**, 245–420.
3. MORGAN, T. H. *Quart. J. Mic. Sci.*, 1895, **37**, 445–459.
4. MORGAN, T. H. *Columbia U. Biol. Ser.*, 1901, **VII**, 9.
5. SMITH, FRANK. *Proc. U. S. Nat. Mus.*, 1917, **52**, 157–182.

Liver Tumors in Rats Fed Thiourea or Thioacetamide

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The striking effectiveness of thiourea and thioacetamide in preventing orange decay (*1–3*) and the occurrence of the thiourea in the juice of treated fruit led us to study the chronic toxicities of these substances. Purves and Griesbach (*5*) observed adenomas of the thyroid glands in rats treated with 0.25% thiourea in their drinking water for 12 months or more. After 20 months of treatment there was a tendency for the tumors to become malignant. Since no neoplastic changes were observed in other tissues than the thyroid glands after this long period of thiourea administration, these observers concluded that thiourea had no direct carcinogenic action. In contrast to the observations of Purves and Griesbach, we found the production of liver tumors to be one of the chronic effects of thiourea (*4*). The present communication reports the nature of these liver tumors and their high incidence in thiourea-fed rats.

In a two-year chronic toxicity study albino rats, 21 days old, 18 to a group, were fed thiourea at levels of 1, 0.5, 0.25, 0.1, 0.05, 0.025, and 0.01% in a diet of ground commercial rat biscuits. Control animals received the basic diet. All animals were permitted unrestricted access to both food and water.

At dosage levels of 0.25% or more thiourea, the outstanding gross lesion was enlargement of the thyroid gland. The thyroid enlargement was marked at the 1.0% level and decreased with decreasing dosage, but was distinct at 0.25% thiourea. The thyroid weights of the animals on dosage levels of 0.05% or lower were not significantly different from those of the controls. Thiourea at dosage levels of 0.25% or more stunted the growth of the rats. This effect was marked during the fast-growing period of the first three months on the experimental diet. When the rats on these higher dosage levels became adult, they were short, chubby, and very fat. They appeared listless and, when disturbed, made no effort to move around in their cages. All animals at the dosage levels of 0.25% or more thiourea died within the first 17 months of the experiment. Lower dosages had no effect on mortality.

The liver showed marked gross changes, especially in the surviving animals, at dosage levels of 0.10% and below. At levels of 1.0 and 0.5% the liver and, to a lesser extent, the viscera in general were in the majority of instances moderately pale. The pallor was not so pronounced at 0.25% and was essentially absent below this level. In no animal was there an hepatic cirrhosis or even a roughness of the liver surface, except as caused by the presence of tumors.

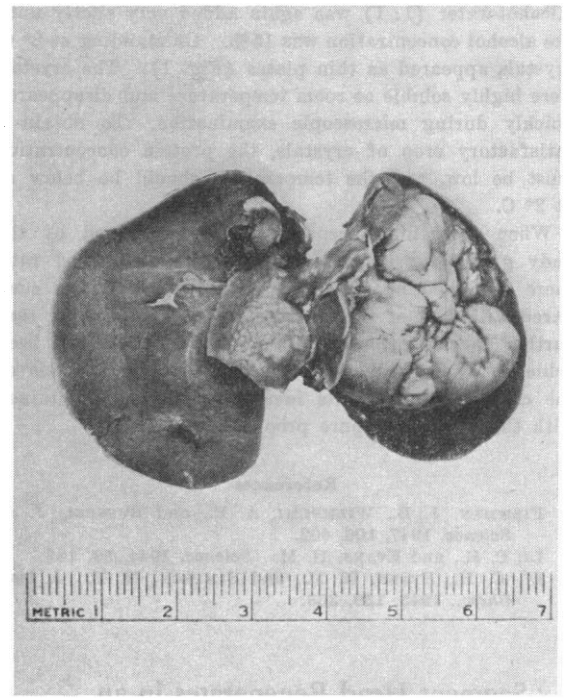

FIG. 1. Large liver tumor in rat fed 0.1% thiourea for two years.

Of the 29 experimental rats surviving the two-year feeding period, 14 showed liver tumors. There was a general correspondence between dosage level and tumor size and incidence. Only one of the nonsurviving treated rats, and none of the 18 controls, showed such a tumor. For comparison, the general run of our rats, whether controls or those fed a variety of added substances in their diets and surviving a two-year experimental period, show approximately a 1% incidence of spontaneous hepatic tumors of the type described below. In the livers of the animals with these spontaneous tumors, also, cirrhosis is absent. The significance, therefore, of an almost 50% tumor incidence in the surviving thiourea-fed animals becomes apparent.

In size, 4 of the 15 liver tumors were $2.5 \times 2.5 \times 2.0$ cm or larger, up to $4.0 \times 3.5 \times 2.5$ cm. One of the larger ones is illustrated in the photograph (Fig. 1). The rest varied from this size down to 4 mm in diameter. In about half of the instances there were, in addition to the main tumor, one or a few smaller nodules, some of which had a distinct tumorous appearance while others were not grossly typical. The tumors were on the whole very similar to each other in appearance, being well circumscribed and sharply demarcated from the surrounding liver because of their lighter color. This was especially true for the larger ones. There was no fibrous encapsulation. All sites in the liver were equally affected.

Microscopic hyperplasia of the thyroid gland paralleled the gross enlargement in a general way, but at any given dosage level was more consistent. At the 1.0% level the hyperplasia averaged marked in degree, at 0.5% moderate to marked, at 0.25% moderate, at 0.10 and 0.05% very slight; at levels below this there was little, if any, hyperplasia. No detailed histologic description of the thyroid hyperplasia seems necessary, since it was essentially identical with that reported by various authors.

The liver showed as its most consistent lesion a mild generalized hypertrophy of the hepatic cells. Accompanying this were slight irregularity of the hepatic architecture, slight (in rare instance, moderate) bile duct proliferation, and minimal degrees of fatty change (as evidenced by vacuolation in the paraffin sections) and hyaline appearance of the hepatic cell cytoplasm. No portal fibrosis was evident. A peculiarity of these hepatic changes was the fact that at any given dosage level there was very little progression or increase in degree between the earliest animals examined and the latest. There was, however, a gradual reduction in degree as the dosage level was decreased, and at 0.05% and below these hepatic alterations were either minimal or absent, except in the near vicinity of some tumors.

The liver tumors were quite uniform in their histology and, except for minor areas of variation, were composed of irregular cords of cells resembling the surrounding hepatic cells. In the smaller tumors the resemblance was rather close, while, as the tumors became larger, their cells also became larger and less typical. The nuclei and nucleoli showed a slight relative prominence as compared to the nonneoplastic hepatic cells. Mitoses were rarely seen. Normal hepatic lobulation was lost even in the smallest tumors. Endothelial-lined sinusoids were generally present between the cell cords, but, as the tumors became larger, the sinusoids became more irregular in size and shape. Generally the tumor borders were sharply demarcated from the nonneoplastic hepatic cells, but there was little or no fibrous encapsulation. Generally the neoplastic masses had an expansive appearance and pushed aside and compressed the adjacent liver. Features present in the tumors in small amount or in slight degree were focal bile duct proliferation, fibrosis, focal necrosis, solid masses of tumor cells, and telangiectasia. With full realization of the difficulties of the nomenclature of hepatic cell masses, we can only say in the limited space available that we consider these tumors as hepatic cell adenomas.

Apart from the thyroid and liver, certain other structures showed changes attributable to the feeding of thiourea and not simply nonspecific accompaniments of inanition. In the spleen and adrenal, these changes were rarely seen at levels of 0.10% and below, but above this level they were nearly consistent, although somewhat less pronounced at 0.25% than at 1.0%. The splenic pulp was markedly atrophic, the follicles less so, and in contradistinction to the usual atrophic spleen the pulp was markedly congested and contained a slight to moderate excess of hemosiderin over the amount ordinarily seen. The reticular zone of the adrenal cortex was congested and atrophic. In the kidney, one-third of the animals at 0.25%, increasing to two-thirds at 1.0%, showed small to moderate numbers of calcified tubular casts at the vicinity of the corticomedullary junction; several of these kidneys also contained small amounts of calcified debris in the pelvis. Reduction or cessation of spermatogenesis, reduction of bone growth at the epiphyseal lines, and hypoplasia of the bone marrow could all be accounted for by the degree of bodily stunting present, but a slight to moderate thickening of the cancellous bone trabeculae in some of the higher-dosage animals could not be so accounted for.

Lung, heart, pancreas, stomach, small intestine, colon, uterus, ovary, parathyroid, lymph nodes, thymus, and voluntary muscles all showed no changes specifically attributable to thiourea.

In a chronic toxicity study similar to that for thiourea, 50 rats, 10 to each group, were fed thioacetamide at levels of 0.1, 0.05, 0.025, 0.01, and 0.005%. At dosage levels of 0.025% and above there was a marked effect on survival time and a significant effect on growth rate. The outstanding lesion was a cirrhosis of the liver. At 0.1% thioacetamide, survival was for less than a month, and there was no gross hepatic cirrhosis; however, the liver of the rats at this level showed microscopically a complicated pattern of damage, two features of which were a moderate degree of bile duct proliferation and a relatively enormous increase in size of the hepatic cell nucleoli. At 0.05% a marked degree of coarsely nodular cirrhosis of the liver was present after the first few weeks, and one of the livers contained a 3.5-cm area of histologically malignant tumor originating from hepatic cells. Such a tumor has never occurred spontaneously in our rats. At 0.025% cirrhosis was slight to moderate in degree, and there were no liver tumors. At 0.01 and at 0.005% there was minimal or no cirrhosis, but 1 of the 6 surviving animals had a 1-cm hepatic cell adenoma. Thyroid, lung, heart, spleen, pancreas, gastrointestinal tract, kidney, adrenal, lymph nodes, gonads, bone marrow, bone, muscle, and parathyroid showed either no changes attributable to treatment or, if there were any, they were so slight as to be questionable.

It is concluded that thiourea, administered orally to albino rats for a prolonged period of time, induces liver tumors, without liver cirrhosis, in a large percentage of

cases at concentrations which may be below those producing hyperplasia of the thyroid gland. Thioacetamide appears to be slightly tumorigenic in the rat liver and, in addition, is a very potent producer of nodular cirrhosis.

References

1. CHILDS, J. F. L., and SIEGLER, E. A. *Phytopath.*, 1944, **34**, 983.
2. CHILDS, J. F. L., and SIEGLER, E. A. *Science*, 1945, **102**, 68.
3. CHILDS, J. F. L., and SIEGLER, E. A. *Ind. eng. Chem.*, 1946, **38**, 82.
4. FITZHUGH, O. G., NELSON, A. A., and HOLLAND, O. L. *Fed. Proc.*, 1948, **7**, 218.
5. PURVES, H. D., and GRIESBACH, W. E. *Brit. J. exp. Path.*, 1947, **28**, 46.

Desoxypentosenuclease in Yeast and Specific Nature of Its Cellular Regulation[1]

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Evidence has been obtained in this laboratory of the occurrence in yeast (*Saccharomyces cerevisiae*) of two agents concerned with desoxypentose nucleic acids (DNA), viz., a depolymerase (DNase) and an inhibitor of DNase having interesting specific properties.

When yeast is ground mechanically and the layer of cell debris treated with M NaCl solution, the extract, as was shown recently (*1*), includes a highly polymerized DNA. The same extract has now been found also to contain a DNase in a largely inhibited state, from which it is slowly released on storage at 4°. The increase in activity is about 50-fold within 3 months. Extracts of the ground cells with distilled water contain free inhibitor but no enzyme.

Washed yeast was crushed, distilled water being used as suspending fluid, and the debris extracted with M NaCl, as described previously (*1*). The viscous mixture (500 cc) was kept in the refrigerator for 1–4 months, clarified by centrifugation at 4,000 rpm, dialyzed, and dried from the frozen state in a vacuum. The solution of the residue in 30 cc of water was centrifuged at 20,000 rpm and the supernatant brought to 0.6 saturation with solid ammonium sulfate. The solution of the precipitate, collected at 20,000 rpm, was subjected to a rocking dialysis against ice-cold distilled water for 7 hrs and again centrifuged at high speed. The sediment was washed with water and then extracted with 30 cc and again with 12 cc of M NaCl. The combined extracts, clarified by centrifugation at 20,000 rpm, were dialyzed and evaporated in a vacuum in the frozen state. The DNase preparation weighed 27 mg. Even high dilutions of this agent produced a rapid drop in viscosity of solutions of thymus DNA and of yeast DNA (*1*). It had an activity of about 1,200 units/mg of protein, as defined by McCarty (*4*). In a concentration of 0.6 mg/cc it was free of proteolytic (*5*), nucleotidase, and ribonuclease activities.

Yeast DNase resembled the desoxyribonuclease of pancreas (*3*, *4*) in requiring Mg ion for activation and in being labile to heat; the activity was destroyed completely by heating to 55° for 15 min. It differed, however, from the pancreatic desoxyribonuclease in several important respects. It was insoluble in water but soluble in salt solutions. Its activity optimum lay below pH 6.2; at pH 8.1 only 20% of its activity was retained. The most significant difference consisted in its being specifically inhibited by a yeast fraction which, however, had no inhibiting effect on purified pancreas desoxyribonuclease[2] and on crude DNase preparations from *Neurospora crassa*, germinating barley, and calf thymus, which will be discussed on a later occasion.

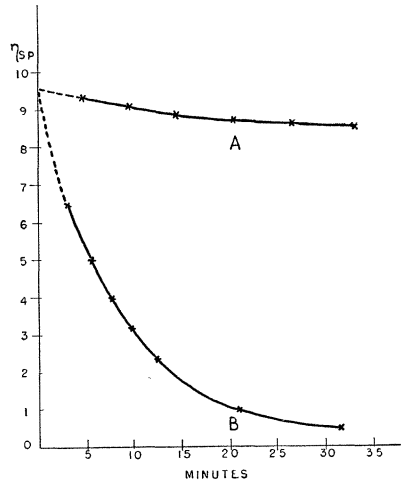

FIG. 1. The specific viscosities of mixtures of thymus DNA (sodium salt) and yeast DNase with (A) and without (B) DNase inhibitor are plotted as the ordinate. The abscissa indicates the duration of incubation of the mixtures before testing, at 30°. Mixture A contained 1.7 mg of DNA, 3 micromoles of Mg, 2.5 units of yeast DNase, and 2.2 mg of inhibitor/cc of veronal buffer of pH 6.6. In mixture B the inhibitor was omitted.

The DNase inhibitor, present in aqueous and salt extracts of ground yeast cells, caused up to 94% inhibition of yeast DNase. A typical experiment is reproduced in Fig. 1. The inhibitor appears to be a water-soluble labile protein, its activity being destroyed in less than 5 min at 55°. It is inactivated by crystalline trypsin, by ficin, and by a proteolytic enzyme preparation from crushed yeast. To the presence of the latter in the inhibitor preparations their inactivation on storage for 8–20 hrs at 30° or for 2–4 weeks at 4° probably is attributable. The inhibitor can be partially purified by precipitation at 0.8 saturation with ammonium sulfate.

[1] This work has been supported by a research grant from the U. S. Public Health Service.

[2] We are very grateful to Dr. M. McCarty for a specimen of this preparation.