

SUMMARY.

1. The production and methods of extraction of antibacterial substances from *Stereum hirsutum* are described.
2. The fungus liberates into the metabolism fluid an inactive extracellular precursor which is activated by an enzyme contained in the mycelium.
3. The inactive precursor can be converted to weakly antibacterial substances, different from the natural antibiotic, by chemical means.
4. Some of the chemical and biological properties of the substances are described.
5. The substances do not appear to have any therapeutic potentialities.

We are indebted to Dr. A. G. Sanders for some of the heart perfusion experiments. We are glad to acknowledge the technical assistance of J. Kent, M. Lancaster and P. Morris.

We have received financial assistance from the Medical Research Council and the Rockefeller Foundation.

REFERENCES.

- ABRAHAM, E. P., CHAIN, E., FLETCHER, C. M., FLOREY, H. W., GARDNER, A. D., HEATLEY, N. G., AND JENNINGS, M. A.—(1941) *Lancet*, ii, 177.
FEARON, W. R., AND MITCHELL, D. M.—(1932) *Analyst*, **57**, 372.
FREED, M., AND WYNNE, A. M.—(1936) *Ind. eng. Chem. (anal.)*, **8**, 278.
HEATLEY, N. G.—(1944) *Biochem. J.*, **38**, 61.
MARTIN, A. J. P., AND SYNGE, R. L. M.—(1941) *Ibid.*, **35**, 1358.

STUDIES ON EXPERIMENTAL GOITRE. VIII: THYROID TUMOURS IN RATS TREATED WITH THIOUREA.

H. D. PURVES AND W. E. GRIESBACH.

From the Thyroid Research Department of the New Zealand Medical Research Council, University of Otago, Dunedin, N.Z.

Received for publication January 7, 1947.

SINCE Bielschowsky (1944) demonstrated a synergistic action between the carcinogen 2-acetamidofluorene and the goitrogenic agent allylthiourea in the production of thyroid neoplasms, interest has been aroused in the role of the goitrogenic agents in inducing thyroid neoplasia. Griesbach, Kennedy and Purves (1945) showed that the administration of a rape seed diet which contains a goitrogenic agent produced thyroid neoplasms in the rat, and considered that the induction of these tumours was due to the long-continued stimulation of the thyroid by the thyrotropic hormone, and not to any specific neoplasia inducing effect of any substance in the rape seed. Bielschowsky (1945) has reported the production of adenomata in the rat thyroid by the administration of the goitrogenic substance allylthiourea alone, though the incidence of such tumours was

small compared with those produced by the combination of allylthiourea and acetamidofluorene. He considered on the basis of histological appearance that these adenomata were not under the same control by the thyrotropic hormone as is normal thyroid tissue.

The present communication reports the result of long-term administration of the goitrogenic substance thiourea. The nature of the thyroid tumours produced and the response of such tumours to fluctuations in thyrotropic stimulation are described.

METHODS.

Some of the rats used were of the local albino strain of *Rattus norvegicus* used in previous experiments. In addition a group of rats of a recent Wistar strain were used. The diet was that previously described as diet S 3 (Griesbach, Kennedy and Purves, 1945). Thiourea was administered as a 0.25 per cent solution in the drinking water. When it was desired to inhibit the thyrotropic hormone secretion, injections of thyroid extract were given without interrupting the thiourea administration. For the administration of the thyroid extract, thyroid powder (B.P. '32) was brought into solution by peptic digestion in 0.2 N-HCl. The solution was then neutralized and diluted till 1 ml. contained 50 µg. of iodine. Injections of 0.2 ml. of this solution were given daily.

The thyroids were dissected out at autopsy, weighed and fixed in formal-saline. Serial sections were cut at 5µ and stained with Harris's haematoxylin and eosin or with Heidenhain's azan method. When adenomata were observed sections were taken at 2.5µ for the purpose of photomicrography.

TABLE I.—*Production of Thyroid Neoplasms by Long-term Thiourea Administration.*

No. of rat.	EXPERIMENT A (Males, local strain).		EXPERIMENT B (Females, local strain).		EXPERIMENT C (Males, Wistar strain).	
	Thiourea treatment (months).	Thyroid neoplasms.	Thiourea treatment (months).	Thyroid neoplasms.	Thiourea treatment (months).	Thyroid neoplasms.
1	11	nil	5	nil	6½	nil
2	16	A	9½	nil	8½	nil
3	16	A	12	A	12	A
4	17	A	12	A	16	A
5	17	A	19½	A	16	A
6	20½	A, C	20	A, C	17	A
7	22½	nil	22½	A, C, F	17	A, F
8	22½	C	23½	A, C, F	21	A
9	23½	A, C, F	23½	A	21	nil
10	23½	A, C	23½	A	21½	nil

A = Adenoma, benign histological appearance.

C = Carcinoma, malignant histological appearance evidenced by invasion of blood vessels.

F = Foetal adenoma.

RESULTS.

Thyroid tumours were observed in a high proportion of the rats which received thiourea for 12 months or longer. Histologically three classes of tumours were found—benign adenomata, adenocarcinomata and foetal adenomata. The incidence of the three types of tumours is summarized in Table I.

I. Production of thyroid adenomata by thiourea treatment.

(a) Ten male rats of the local strain were placed on thiourea at the age of 8 weeks. One of these died from an intracranial abscess 11 months from the commencement of the experiment. No adenomata were seen in the thyroid of this animal. The remaining nine animals were sacrificed at times ranging from 16 to 23½ months after the commencement of the experiment. In one of these no thyroid neoplasm could be found on histological examination. In the remaining eight animals one or more thyroid tumours were found. In one animal (No. 8 of Exp. A, Table I) the single neoplasm found was relatively malignant in histological appearance. In three other animals tumours of relatively benign and relatively malign appearance were coexistent. In the remaining four animals only benign looking tumours were observed.

(b) Ten female rats of the local strain were placed on thiourea at the age of 8 weeks. Two which died at the age of 5 and 9½ months had no thyroid tumours. The remaining eight animals were killed at various times after 12 to 23½ months' thiourea treatment. All eight had benign looking thyroid neoplasms detected by histological examination. Three of these rats had, in addition, malignant looking thyroid tumours.

(c) Ten male rats of a recent Wistar strain were placed on thiourea at the age of 8 weeks. In two which died after 6½ and 8½ months of thiourea treatment no tumours were found on histological examination. Six of the remaining eight animals had benign looking adenomata. No tumours were found in two animals killed at 21 months after the experiment had been started. None of the growths in the six tumour-carrying animals was classified as malignant.

II. Malignancy.

Up to 19½ months from the beginning of the experiment the tumours were similar in appearance to those previously seen and described by us in rats on Brassica seed diet (Griesbach, Kennedy and Purves, 1945). These tumours were benign in histological appearance. At the 20th month one rat was found to have a benign looking tumour in one thyroid lobe, while in the other lobe there was a tumour with histological evidence of malignancy. Of the thirteen animals which were killed after 20 months' or more thiourea treatment, three were without thyroid tumours. In the ten tumour-bearing rats seven tumours were found with frankly carcinomatous histological appearance. The main criterion of malignancy was invasion of the walls of blood vessels. In two of these animals the malignancy of the tumours was confirmed by the finding of metastases in the lungs. The appearances in these animals have been described in a previous publication (Purves and Griesbach, 1946).

III. Foetal adenoma.

In three instances nodules of solid cellular growth were found, which we consider to correspond closely to what is called "foetal adenoma" in human pathology. One of these nodules grew in the centre of a benign looking adenoma (Fig. 11 and 12), while each of the remaining two nodules, one of which has been mentioned in an earlier paper (Purves and Griesbach, 1946), appeared within an adenocarcinoma of the thyroid (Fig. 10). In every case the boundaries between the two kinds of tissue were distinct. The foetal adenoma nodules consisted of

solid masses of cells with large vesicular nuclei, poorer in chromatin than the surrounding tissue. There was a trabecular structure recognizable in one of these tumours, but no colloid formation could be seen, not even after three weeks of thyroid digest injections. Mitotic figures were observed in these foetal adenomata. No tendency to invade blood vessels was apparent, and no lung metastases of "foetal adenoma" structure were found.

IV. Response of thyroid tumours to thyrotropic hormone.

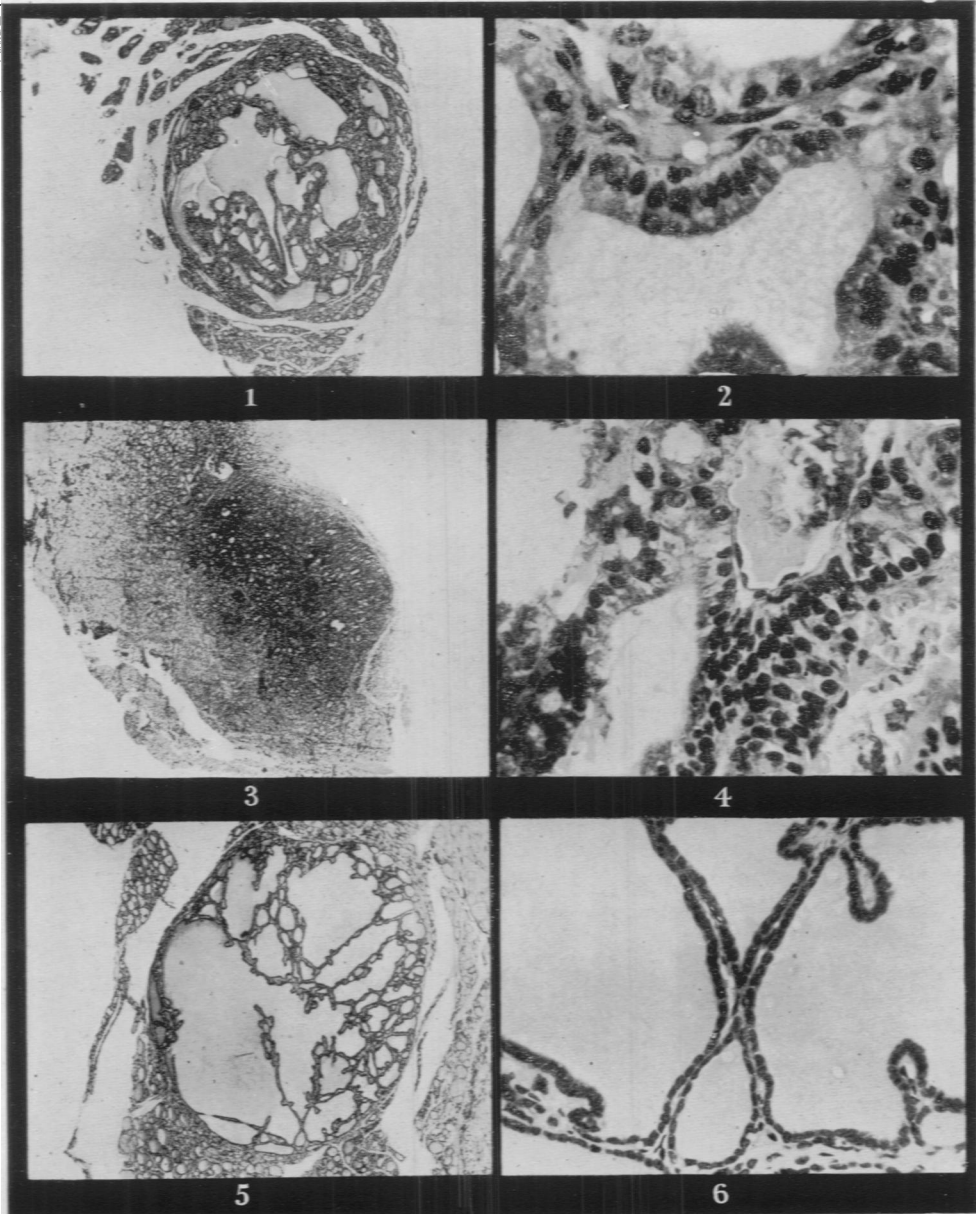
In order to study the response of the thyroid adenomata to the thyrotropic hormone, an experiment was made in which injections of thyroid active material were made to inhibit the secretion of thyrotropic hormone by the pituitary. The thyroid preparation used was a peptic digest of standard Thyroid B.P. '32, the preparation of which is described above. The injections of 0.2 ml. daily contained 10 micrograms of iodine per injection, which would contain about 5 micrograms of 1-thyroxine. This is in excess of the daily requirement of the rat, and causes inhibition of thyrotropic secretion by the pituitary (Griesbach and Purves, 1945). The administration of the thiourea in the drinking water was continued throughout the experiment. Eight rats were used, four being chosen for the purpose from each of the experimental groups A and C, which at this time had received 16 months' thiourea treatment. Four rats were killed after 21 days of thyroid digest injections. The thyroid digest administration was then stopped, and the remaining four rats killed after a further 28 days' treatment with thiourea alone. All eight rats were found at autopsy to have extensive thyroid adenomata. The histological appearance of the adenomata in the four rats killed after 21 days of thyroid administration was profoundly altered compared with that of tumours before thyroid administration. The large spaces which, as we have pointed out, are formed by the coalescence of acini, were distended with dense, deeply-staining colloid forming large colloid lakes, quite unlike any appearance of the normal rat's thyroid. The epithelium of these adenomata was markedly inactive compared with the appearance of any of the adenomata of this series before thyroid administration, or of any of those previously described as occurring during rape seed administration. One of these adenomata, illustrated in Fig. 7 and 8, showed in places an extreme degree of flattening of the epithelium. In another adenoma the epithelium was cuboidal in structure, but differed from the cuboidal epithelium of normal thyroid activity in that the cytoplasm was extremely scanty and the nuclei were closely packed together (Fig. 5 and 6). This close packing of the cells, which is a characteristic of the adenomata before thyroid treatment (Fig. 2 and 4), does not allow of flattening of the epithelium except where distension by colloid accumulation has stretched the acinar walls and allowed space for lateral expansion of the cells (Fig. 8). In the four rats which received thiourea alone for 28 days after cessation of thyroid injections, the thyroid adenomata, like the surrounding normal thyroid tissue, showed histological evidence of reactivation. The cells of the adenomata were hypertrophic, as shown in Fig. 9, and were quite as active in this respect as the cells of thyroid adenomata before thyroid treatment. Colloid, however, remained in considerable amount in these reactivated tumours, and was clearly in excess of that present in thyroid adenomata which had not been subjected to the inactivating influence of thyroid injections. It was, however, distinctly thinner

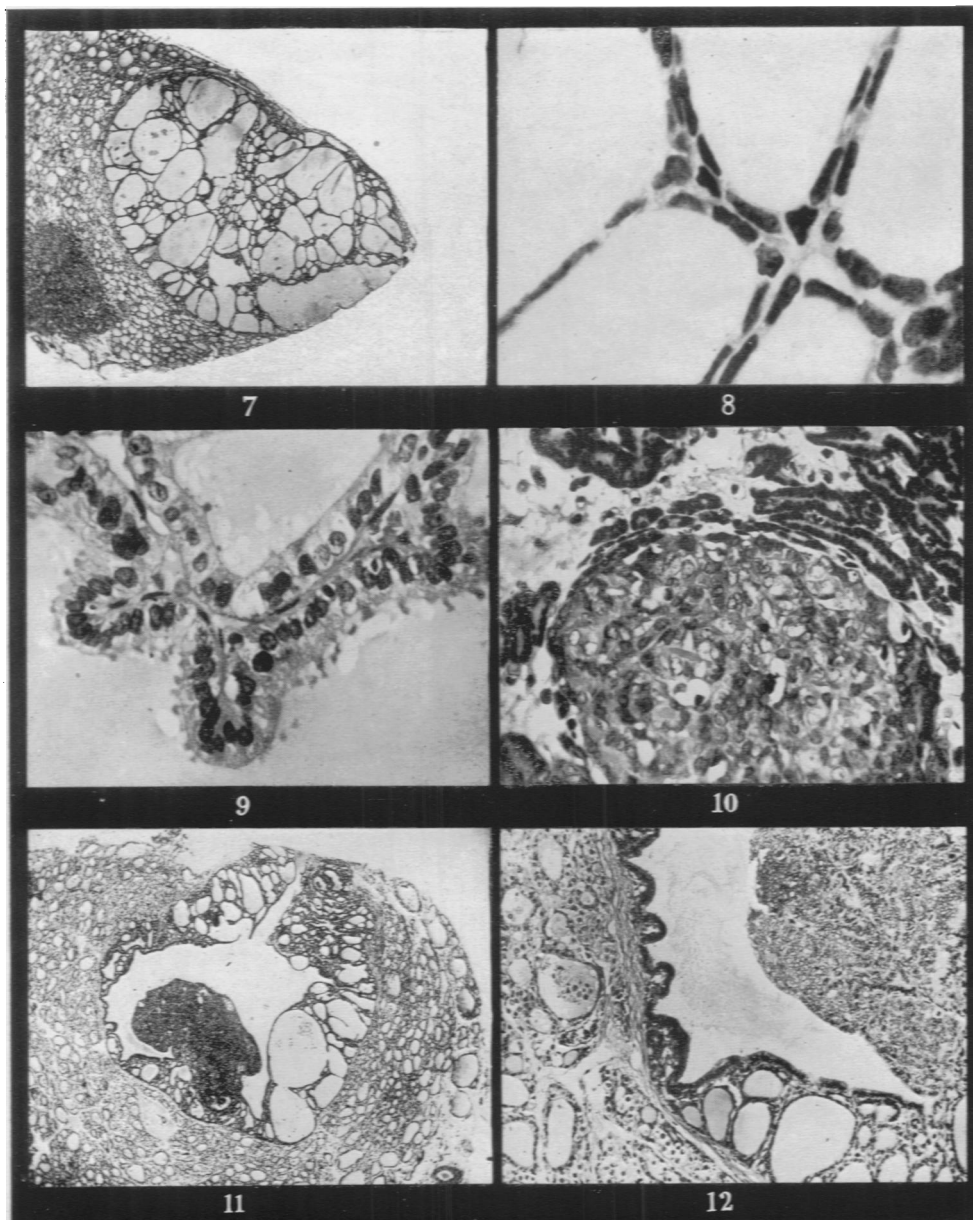
than in those tumours obtained immediately after the 21 days' thyroid treatment, so that colloid absorption was evidently taking place, although at a slower rate than in normal thyroid tissue.

After 22½ months of thiourea administration the remaining five surviving rats were treated with thyroid digest by injection for a period of 3 weeks. When these animals were killed, two were found to have the benign looking type of adenoma in the thyroid, and these tumours were involuted, with colloid storage and flattened epithelium. The other three animals showed thyroid tumours classified as malignant on the basis of histology. The cells of these adenocarcinomata were involuted by comparison with those of any of the non-thyroid-treated tumours. The nuclei were small and pyknotic and the cytoplasm was very scanty. In certain areas acinar spaces filled with dense colloid were present, and in these areas the appearance was typical of the involuted benign adenoma. In other areas the epithelium was arranged in cords or trabeculae without acinar formation, and in these spaces there was no colloid accumulation. The lung metastases found in one of these animals contained well-formed acini which were distended with colloid.

DESCRIPTION OF PLATES.

- FIG. 1.—Thyroid of rat after 16 months' thiourea treatment. A single adenoma with cystic spaces containing dilute colloid. A small amount of compressed normal thyroid tissue surrounds the adenoma. H. & E., × 34.
- FIG. 2.—High-power view of adenoma in Fig. 1. The tall columnar cells are clearly shown. The acinar space contains a small amount of coagulated colloid. H. & E., × 650.
- FIG. 3.—Thyroid of rat after 16 months' thiourea treatment. The adenoma is highly cellular, and stains more deeply than the surrounding normal tissue. H. & E., × 19.
- FIG. 4.—High-power view of part of adenoma in Fig. 3. In the upper right-hand corner is a blood-filled space lined by endothelium only. The cells are mainly columnar, and the acinar spaces are empty. H. & E., × 650.
- FIG. 5.—Thyroid of rat after 16 months' thiourea treatment, followed by 3 weeks' treatment with thyroid digest. The cystic spaces are full of dense colloid. Colloid accumulation can be seen in the acini of the adjacent thyroid tissue. Before thyroid treatment this adenoma was probably similar to that of Fig. 1. H. & E., × 30.
- FIG. 6.—High-power view of part of Fig. 5. The involution of the cells is evident by their scanty cytoplasm in comparison with Fig. 3. The close spacing prevents any further flattening of the cells. H. & E., × 270.
- FIG. 7.—Thyroid of rat after 16 months' thiourea treatment, followed by 3 weeks' treatment with thyroid digest. An adenoma is shown with acinar spaces greatly distended by colloid accumulation. The dark mass in the lower right corner is the parathyroid. H. & E., × 30.
- FIG. 8.—High-power view of part of Fig. 7. The cells have scanty cytoplasm and show an extreme degree of flattening. H. & E., × 650.
- FIG. 9.—High-power view of an adenoma which has been reactivated by 4 weeks of thiourea treatment after 3 weeks of treatment with thyroid digest. The cells are tall columnar, comparable with those of Fig. 2, and contrast strikingly with those of Fig. 6 and 8. There has been a partial resorption of colloid. H. & E., × 650.
- FIG. 10.—Adenocarcinoma in a rat which received thyroid digest for 3 weeks before death. On the left are darkly staining adenocarcinoma cells. By comparison with Fig. 4 these cells are seen to be markedly involuted. The acinar spaces contain colloid but are not distended. On the right is a nodule composed of pale stained cells showing no acinus formation. This nodule is considered to be a foetal adenoma. H. & E., × 650.
- FIG. 11.—Cystic adenoma in thyroid of a rat after 17 months' thiourea treatment. A foetal adenoma forms a solid cellular growth projecting from the wall of the cyst. H. & E., × 30.
- FIG. 12.—Part of Fig. 11 at higher magnification. The pale stained cells of the foetal adenoma in the top left-hand corner are arranged in cords. The wall of the cyst is composed of tall columnar epithelium, which is deeply stained and quite distinct from the cells of the foetal adenoma. H. & E., × 144.





DISCUSSION.

The production of thyroid adenomata by the administration of thiourea is not unexpected, in view of the previous finding of thyroid adenomata produced by rape seed administration and the production of adenomata by allylthiourea administration reported by Bielschowsky (1945). In contrast to the rapid appearance of the thyroid tumours in rats treated simultaneously with acetamidofluorene and allylthiourea, as reported by Bielschowsky (1944), under the influence of the goitrogen alone thyroid tumours appeared only after a considerable delay. Once the tumours appear they apparently grow rapidly, and it therefore seems that tumours are initiated only after many months of treatment with the goitrogenic agent. In those animals which were treated for one year or more thyroid tumours were found in twenty-two out of twenty-five, giving an incidence of 88 per cent. In our experiments there was no certain indication of any difference in susceptibility of the two sexes or of the two strains which were tested.

Of the twenty-two animals which had thyroid tumours, seven showed tumours classifiable as malignant on histological appearances, giving an incidence of 31 per cent. The first of these malignant tumours was observed after 20 months. In the thirteen animals which were treated with thiourea for 20 months or more there were seven malignant tumours, an incidence of 54 per cent. The fact that the malignant tumours were always relatively large and in most cases were associated with smaller tumours of benign appearance situated in the other thyroid lobe leads to the conclusion that the early adenomata have taken on a malignant appearance under continued stimulation. However, there was nowhere any suggestion that a small nodule of malignant growth had appeared in a larger benign tumour. The change to the malignant appearance seems to be the result of an evolution in the growth of the whole tumour. The fact that Wegelin (1927) and Hellwig (1935) observed similar tumours in the thyroids of rats on iodine-deficient diets suggests that the tumours produced by goitrogenic agents are not the result of any carcinogenic action of the thioureas, but are the result of long-continued intense stimulation by the thyrotropic hormone. The fact that lung metastases were observed in some of our rats is not proof that these thiourea-induced tumours are more malignant than those resulting from iodine deficiency, since such lung metastases may easily be overlooked. Moreover, the fact that no neoplasia-inducing effects were observed in tissues other than the thyroid after this long period of thiourea administration is evidence against any direct carcinogenic action of the thiourea.

In three of the thyroid tumours observed in this series of experiments there were structures present which we have called foetal adenomata. These foetal adenomata have clearly arisen in the interior of the pre-existing adenoma or adenocarcinoma. In two cases the parent tumour was classified as adenocarcinoma. The interesting thing is that by secondary change a tumour of benign behaviour has arisen from one of malignant character. The association of foetal adenoma with malignant thyroid tumour in human thyroids has led some pathologists to the view that foetal adenoma is potentially malignant and may give rise to adenocarcinoma. Our observations of these early tumours in the rat suggests that the adenocarcinoma may well have antedated the foetal adenoma. The preconceived idea that benign neoplasms commonly give rise

to malignant growths by secondary changes needs careful consideration, since these studies suggest that, even where benign and malignant structures co-exist, it is by no means certain that the malignant structure has been derived from the benign. In these thyroid tumours there was never any evidence of a more malignant structure arising inside a pre-existing benign structure. In some tumours which were mainly follicular in structure there were areas where the cells were arranged in cords and trabecules without acinar formation. The idea that these areas might be a more malignant variation of the primary tumour is, however, negated by the evidence of the metastases in the lung, where well-formed acini were found.

The thyroid neoplasms described in this paper were all responsive to fluctuations in the level of thyrotropic hormone. Indeed, in the case of the benign adenomata, colloid accumulation after administration of thyroid substance was much more marked than in the surrounding normal thyroid tissue. These involuted adenomata can be reactivated, and respond with a prompt cellular hypertrophy, though the resorption of the colloid is much slower than in the normal thyroid. This readiness to accumulate colloid and reluctance to discharge it is related to the tendency noted by Bielschowsky (1944, 1945) for these adenomata to contain even under conditions of stimulation a certain amount of colloid in contrast to the normal hyperplastic thyroid where colloid is almost entirely absent. This effect may be related to the large size of most of the acinar spaces, which results in the ratio of cellular surface to acinar volume being relatively less in the adenomata. The supply of proteolytic enzymes by which the solution of the colloid is effected is therefore less in the adenomata.

The "foetal adenoma" type of tissue did not show any reaction after thyroid administration. The cell nuclei were large and pale, the cytoplasm was well defined and there was no colloid accumulation.

The three tumours of carcinomatous type which had not been under thyrotropic stimulation for 21 days showed a definite epithelial change towards regression. The nuclei were pycnotic and showed the signs of mutual compression. The cytoplasm was hardly recognizable. In parts with trabecular configuration there was no colloid storage visible, but where a more acinar structure prevailed large colloid-filled vesicles were present. It is evident that malignant behaviour in thyroid tumours is not necessarily accompanied by escape from thyrotropic control. It may therefore be possible to influence the course of malignant thyroid disease in human beings by the therapeutic administration of desiccated thyroid.

SUMMARY.

1. Prolonged administration of thiourea to rats caused the formation of thyroid adenomata. In the second year of thiourea treatment 88 per cent of the animals showed adenoma formation. No significant sex difference or strain difference in susceptibility was observed.

2. After 20 months of treatment seven out of thirteen of these tumours showed the histological appearance of adenocarcinoma. In all seven cases invasion of the blood-vessel walls was visible. In two of these cases lung metastases of thyroid tissue were found.

3. In three thyroid tumours, two of which were definitely malignant, solid masses of cells were found which resembled the "foetal adenoma" of human

pathology. These growths showed benign behaviour. It is considered that these growths were derived from the cancerous tissue.

4. The thyroid adenomata observed in these experiments retained in full their ability to respond to fluctuations in thyrotropic stimulation. Inhibition of pituitary thyrotropic secretion was followed by prompt cell involution and marked colloid storage. When the thyrotropic stimulation was resumed there was a prompt cellular hypertrophy and signs of colloid resorption. The adenocarcinomata also showed full cell involution after withdrawal of thyrotropic hormone and stored colloid in such parts as had an acinar structure. In those parts showing only a trabecular appearance, withdrawal of thyrotropic hormone caused cell involution without producing colloid storage.

REFERENCES.

- BIELSCHOWSKY, F.—(1944) *Brit. J. exp. Path.*, **25**, 90.—(1945) *Ibid.*, **26**, 270.
GRIESBACH, W. E., KENNEDY, T. H., AND PURVES, H. D.—(1945) *Ibid.*, **26**, 18.
GRIESBACH, W. E., AND PURVES, H. D.—(1945) *Ibid.*, **26**, 13.
HELLWIG, C. A.—(1935) *Amer. J. Cancer*, **23**, 550.
PURVES, H. D., AND GRIESBACH, W. E.—(1946) *Brit. J. exp. Path.*, **27**, 294.
WEGELIN, C.—(1927) *Schweiz. med. Wchnschr.*, **8**, 848.

INVESTIGATION INTO THE PRODUCTION OF BACTERIOSTATIC SUBSTANCES BY FUNGI. PRELIMINARY EXAMINATION OF THE SIXTH 100 SPECIES, MORE BASIDIOMYCETES OF THE WOOD-DESTROYING TYPE.

W. H. WILKINS.

From the Mycology Laboratory, University Department of Botany, Oxford.

Received for publication January 9, 1947.

THE present paper, an extension of the series which has appeared from this Laboratory since 1942, is a continuation of the work on basidiomycetes. It follows directly the line of its predecessor (referred to subsequently as "the previous paper") on "the fifth 100 species" (Wilkins, 1946a), and deals in similar manner with another hundred of the same sort of fungi. Further papers on basidiomycetes, the group on which work is at present concentrated, are in course of preparation.

As in the previous paper, the cultures were obtained from the Forest Products Research Laboratory (acknowledgments as before,) and, for the purposes of strain identification, the Laboratory Number is given to each species.

Each fungus was grown on malt and potato dextrose agar, and tested by the "strip" method (Wilkins and Harris, 1944b) against *Bacterium coli* and *Staphylococcus aureus*. In view of previous experience, the positive or negative result of this test was taken as sufficient evidence that the fungus did or did not produce