pound from the animal body due to its increase in molecular weight, but in addition the protective effect of the immune sera to counteract the destructive action of penicillinase normally present in the animal body. It is believed that this is an entirely new approach to the important problem of the delayed action of penicillin. Studies are now in progress along this line and will be published at a later date.

SUMMARY

(1) A combination of penicillin and immune plasma protein has been obtained which possesses bacteriostatic activity.

(2) The presence of the penicillinase immune plasma protein in this mixture protects penicillin *in vitro* from destruction by penicillinase.

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THE EFFECT OF THIOURACIL ON THE RESPIRATION OF BONE MARROW AND LEUCOCYTES *IN VITRO*

THE widespread use of thiouracil in the treatment of hyperthyroidism carries with it the hazard that serious leucopenia or even fatal agranulocytosis may occasionally occur suddenly and unexpectedly during the course of therapy. This danger was pointed out in Astwood's original paper[1] and has been amply confirmed by subsequent reports.[2] It consequently seemed desirable to determine whether thiouracil has any demonstrable effect on the respiratory metabolism of the bone marrow of an experimental animal and to investigate the possibility of combating any depressant action which might be found.

Rabbit femoral bone marrow was employed, the techniques for handling this tissue for measurement of respiration in the Warburg apparatus having been previously worked out.[3] The medium used was autogenous partially neutralized serum, with and without added thiouracil in final concentration of 100 mg per cent. This concentration is much higher than that in the serum of patients being treated with the drug, but it has been shown[4] that in persons receiving thiouracil in therapeutic amounts the drug is highly concentrated in the bone marrow, reaching levels comparable with the above. Most of the determinations were made in triplicate, the others in duplicate. The results reported below are based on the rates of respiration found during the third hour of 3-hour experiments. Differences of ± 5 per cent. between the average rates of respiration of the control samples and those in presence of thiouracil were interpreted as being within the limits of experimental error. Marrows of various cellular composition were obtained by injecting the animals from 3 to 12 days earlier with (a) acetylphenylhydrazine intraperitoneally to produce erythroid metaplasia, or (b) croton oil intrapleurally[5] to produce myeloid metaplasia, previous experience having indicated that neither of these drugs affects the respiratory metabolism of the marrow cells. In each experiment, the proportion of myeloid and erythroid cells present was determined by making differential cell counts on marrow smears stained with Wright-Giemsa.

The results have been found to depend largely upon the cellular composition of the marrow. Of 10 predominantly erythroid marrows (< 40 per cent. myeloid cells) only 3, or 33 per cent., showed a small depression of respiration averaging 9 per cent. ± 2.3 per cent. in the presence of thiouracil. Of 13 marrows in an intermediate group (composition between 40 per cent. and 60 per cent. myeloid cells) 5, or 38 per cent., showed a depression of respiration averaging 10 per cent. ± 1.0 per cent., while of 14 predominantly myeloid marrows (> 40 per cent. myeloid cells) 13, or 93 per cent., showed a depression of respiration that averaged 13 per cent. ± 1.3 per cent. This more uniform and slightly greater susceptibility of the myeloid cells to the depressant effect of thiouracil on cellular respiration led us to determine the effect of the drug on the cells of rabbit peritoneal exudates, since these are virtually all polymorphonuclear leucocytes.[6] In each of 4 experiments in which these cells were washed and resuspended in the same type of media used for the marrow experiments, thiouracil was found to depress respiration, but the extent of the depression (12.9 per cent. ± 1.1 per cent.) was almost identical with that found in "predominantly myeloid" marrows described above. The cell counts of these marrows averaged only 66 per cent. myeloid cells, and since the remaining erythroid cells have been shown to be considerably less susceptible to the action of the drug, the inference is that myeloid marrow cells (mostly myelocytes) are more sensitive to the action of the drug than the mature polymorphonuclear cells found in exudates. Direct

[1] E. B. Astwood, *Jour. Am. Med. Asn.*, 122: 78, 1943.
[2] J. Kahn and R. P. Stock, *ibid.*, 126: 358, 1944; I. Ferrer, D. N. Spain and R. T. Cathcart, *ibid.*, 127: 646, 1945; S. L. Gargill and M. F. Lesses, *ibid.*, 127: 890, 1945.
[3] C. O. Warren, *Am. Jour. Physiol.*, 128: 455, 1940; *ibid.*, 131: 176, 1940; *Jour. Biol. Chem.*, 156: 559, 1944.
[4] R. H. Williams, G. A. Kay and B. J. Jandorf, *Jour. Clin. Inv.*, 23: 613, 1944.
[5] C. O. Warren, *Cancer Research*, 3: 621, 1943.
[6] E. Ponder and J. MacLeod, *Jour. Exp. Med.*, 67: 839, 1938.

observations were also made, on a warmed microscope stage, of the motility of the exudate cells and the myelocytes in the marrow. Thiouracil in 100 mg per cent. concentration did not appear to affect the motility of either type of cell.

Finally, 13 attempts to protect the marrow and exudate cells from the depressant action of thiouracil on respiration by adding 10 mg per cent. pyridoxine[7] *in vitro* all yielded negative results, as have 4 preliminary experiments with diluted liver extract.

A detailed account of these experiments will be published elsewhere.

SUMMARY AND CONCLUSIONS

Thiouracil in 100 mg per cent. concentration induces a small but significant inhibition of respiration of rabbit bone marrow cells, the effect upon the myeloid elements being the more striking. By comparing the results with those obtained with the polymorphonuclear cells of rabbit peritoneal exudates, it is inferred that the more immature marrow cells are more seriously affected. No effects on cell motility have been observed, and attempts to oppose the effect of thiouracil with pyridoxine have been unsuccessful. While caution must necessarily be exercised in relating the results of the present *in vitro* experiments to the known toxic effects of thiouracil in patients, the methods outlined above might be employed to test possible toxic effects of new therapeutic drugs, or the action of agents proposed to protect the marrow from harmful effects.

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ANTAGONISTIC ACTION OF A RED MOULD PIGMENT AGAINST BACTERIA OF THE TYPHOID-PARATYPHOID-DYSENTERY GROUP

A MOULD which produces a red pigment was isolated in our laboratory from human hair planted on Sabouraud's agar.

The mould grows rapidly at room temperature, spreading within 3 to 4 days over the whole surface of an agar plate. At first, the colony is fluffy and pure white; later, within 2 to 3 weeks, it becomes slightly yellowish. A striking characteristic of the mould is the production of a dark red pigment readily diffusing in the medium. No fruiting structures were observed regardless of medium or conditions of growth. The absence of these structures made impossible the identification of the mould.[1] However, it bears no resemblance to any of the known pathogenic fungi. Essential for the formation of the pigment is an organic source of nitrogen (peptone, proteose, casein, bran) and the presence of one of the sugars (dextrose, sucrose, maltose). No pigment is formed on media containing lactose or starch or on Czapek-Dox medium which is used for Penicillium cultures and contains inorganic nitrogen compound. The following medium gives a satisfactory production of pigment: Proteose 1.0, Dextrose 4.0, Agar 0.25, Water 100.0.

The medium is distributed in flasks or bottles in shallow layers 1.5 to 2 cm deep. Three or four days after inoculation, a compact white felt of mycelium develops on the surface of the medium. The formation of pigment which diffuses in the medium begins on the second or third day and attains its peak about the eighth to the tenth day. The pigment can be extracted from the medium by the method used in production of penicillin. After acidification with phosphoric or hydrochloric acid to pH2 the culture medium is shaken with an equal volume of an organic solvent, like ether, amylacetate, butyl alcohol, chloroform. From this solvent the pigment is reextracted by shaking with a phosphate buffer solution of pH7.

The activity of the pigment solution was tested by the Oxford cup method. When typhoid, paratyphoid or dysentery (strains of Shiga and Flexner) bacilli not susceptible to the action of penicillin were seeded on agar plates there always appeared a clear zone of complete inhibition of growth around the cup placed on the surface of agar and filled with the pigment solution. The diameter of the clear zone was 20 to 30 mm. On the other hand, gram-positive bacteria, sensitive to penicillin, such as staphylococci, streptococci, pneumococci and subtilis, were in no way affected by the pigment.

Cultures of the mould grown on media which did not produce pigment did not show antibiotic action—a finding which supports the assumption that the antibiotic properties are intimately connected with the pigment.

The pigment solution is stable and does not lose its antibiotic property after autoclaving, acidification (to pH2) or alkalinization (pH10).

After intravenous injection into a rabbit the pigment, within several minutes, appears in the urine from which it may be recovered in the usual way (acidification, extraction with organic solvent, reextraction with a buffer phosphate solution).

The pigment is not affected by the gastric secretion. When introduced by a tube into the stomach of a

[7] M. M. Cantor and J. W. Scott, SCIENCE, 100: 545, 1944.

[8] These studies were aided by a grant from the John and Mary R. Markle Foundation.

[1] This fact was confirmed by Dr. E. Muskatblit, of New York University, and by K. B. Raper, of the Northern Regional Research Laboratory, U. S. Department of Agriculture, to whom Dr. Muskatblit sent the culture for identification. To both I wish to express my gratitude.