sociation of American Geographers; Raye R. Platt, American Geographical Society; Charles H. Behre, Jr., Society of Economic Geologists; Monroe G. Cheney, American Association of Petroleum Geologists; L. H. Adams, American Ceramic Society; John A. Fleming, American Geophysical Union; *Representative of the Government designated by the President of the United States,* W. E. Wrather; *Members at Large,* W. Storrs Cole, Lester E. Klimm and William W. Rubey.

SPECIAL ARTICLES

THE *IN VITRO* PROTECTION OF PENICILLIN FROM INACTIVATION BY PENICILLINASE

RECENTLY the authors reported a method for the production of an anti-penicillinase immune serum.[1] As readily as the acquisition of immune sera would permit, the investigations were extended to evaluate the therapeutic utilization of such a preparation.

Chow and McKee[2] demonstrated the delayed action of penicillin by combining it with human plasma proteins. They further state that this penicillin-albumin complex, unlike the sulfonamide-albumin complex believed not to possess bacteriostatic activity,[3] does demonstrate antibiotic activity. It was therefore believed that a penicillin-immune plasma protein complex would possess bacteriostatic activity and what is probably more important, also offer protection to the penicillin from destruction by penicillinase.

EXPERIMENTAL

Normal rabbit serum and penicillinase immune rabbit serum were added in varying amounts to a solution containing 4,000 units of penicillin. The volume was restored to 2 cc and after 6 hours' contact, with occasional shaking at 5° C, penicillinase (purity-380 units/mgm) was added in varying amounts, the volume restored to 5 cc, shaken well and incubated at 37° C for one hour. The samples were removed, cooled in an ice bath and immediately assayed by the agar cup plate method.

Experiments were made at two levels of penicillin, with a more pronounced effect at the lower level.

It was found that under the conditions of the experiments, 4,000 units of penicillin were protected from inactivation by as high as 100 units of penicillinase through the addition of 0.25 cc of immune serum. In the control series with normal serum or saline no protection was afforded and less than 25 units of penicillinase were needed for complete inactivation of the penicillin in one hour at 37° C.

TABLE 1

	Serum volume (ml)	Penicillinase units	Penicillin (Oxford units)	Activity (after 1 hour)
Normal serum	0.25	5	4,000	+
	0.25	10	"	+
	0.25	15	"	+
	0.25	20	"	+
	0.25	25	"	0
	0.25	30	"	0
	0.5	5	"	+
	0.5	10	"	+
	0.5	15	"	+
	0.5	20	"	+
	0.5	25	"	0
	0.5	30	"	0
Immune serum	0.25	70	"	+
	0.25	80	"	+
	0.25	90	"	+
	0.25	100	"	+
	0.25	110	"	0
	0.5	80	"	+
	0.5	90	"	+
	0.5	100	"	+
	0.5	110	"	+
	0.5	120	"	0

TABLE 2

	Serum volume (ml)	Penicillinase units*	Penicillin (Oxford units)	Activity (after 1 hour)
Normal serum	0.25	1	1,000	+
	"	3	"	+
	"	5	"	0
	"	7	"	0
	"	9	"	0
	"	11	"	0
Immune serum	"	25	"	+
	"	30	"	+
	"	35	"	+
	"	40	"	+
	"	45	"	+
	"	50	"	+
	"	55	"	0
	"	60	"	0

* A unit of penicillin[4] is that amount of enzyme which in 11 ml of pH 7.0 solution containing 50 Oxford units of penicillin will destroy in one hour at 37° C an amount of penicillin equal to 57.5 per cent. of the penicillin recovered in the control.

In Table 2 it will be noted that the protective effect of immune sera for penicillin is exaggerated by the use of less penicillin (1,000 units instead of 4,000 units) under the same test conditions. It was found that 1,000 units of penicillin was protected from inactivation by as high as 50 units of penicillinase through the addition of 0.25 cc of immune serum, whereas in the control series with normal serum less than 5 units of penicillinase was required for complete inactivation of the penicillin in one hour at 37° C.

It is not known whether a true chemical compound is formed by the addition of penicillin to immune plasma protein, but should this be the case, there would result not only a slower excretion of this com-

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[3] B. D. Davis, SCIENCE, 95: 78, 1942.

[4] E. B. McQuarrie, A. J. Liebmann, R. G. Kluener and A. T. Venosa, *Archiv. Biochem.*, 5: 307, 1944.

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

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