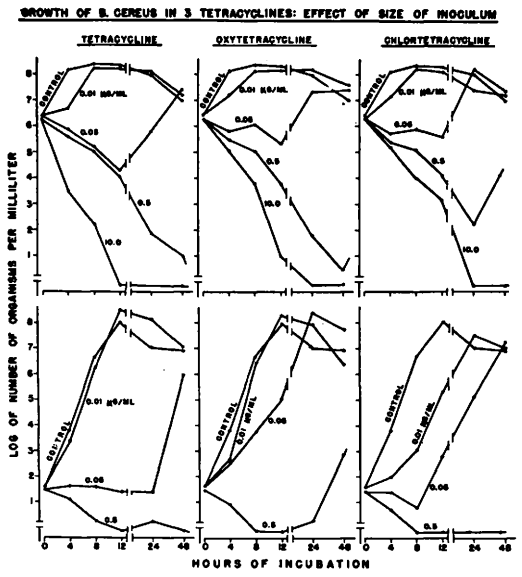

FIG. 3.

very similar under the conditions employed. In the case of individual strains, however, there were significant quantitative differences. The instability of chlortetracycline in the usual *in vitro* tests is well known. The effect of pH was studied and further differences were noted; these have varied somewhat when different organisms were used and the results are not presented since they require further study. In general all 3 agents were more active in an acid medium; this was particularly true of chlortetracycline which was the least active in an alkaline range, at which it was also the most unstable.

In the present study, recently isolated strains were used. This is important because of the increasing incidence of strains of certain organisms which are resistant to both oxytetracycline and chlortetracycline, presumably as a result of the wide use of these agents(5). In the tests for sensitivity, strains resistant to one of the tetracyclines were usually resistant in about the same general range to the other 2 analogues. In a separate study(6), a group of organisms made resistant *in vitro* by repeated subcultures in increasing concentrations of tetracycline and of oxytetracycline were subsequently tested and found to have acquired resistance to all 3 analogues to about the same degree.

Conclusions. The antibacterial action of tetracycline, oxytetracycline and chlortetracycline *in vitro* appeared to be very similar although significant quantitative differences are noted in the action of these 3 analogues on individual strains and under certain cultural conditions.

1. Boothe, J. H., *et al., J. Am. Chem. Soc.,* 1953, v75, 4621.
2. Conover, L. H., *et al., ibid.,* 1953, v75, 4622.
3. Waller, C. W., *et al., ibid.,* 1952, v74, 4981.
4. Stephens, C. R., *et al., ibid.,* 1952, v74, 4976.
5. Finland, M., and Haight, T. H., *A. M. A. Arch. Int. Med.,* 1953, v91, 143.
6. Wright, S. S., and Finland, M., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 40.

Received November 30, 1953. P.S.E.B.M., 1954, v85.

Effect of Total Body X-Irradiation on the Tryptophan Peroxidase Activity of Rat Liver. (20775)

JOHN F. THOMSON AND ELEANOR T. MIKUTA.
From the Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill.

Knox has shown that the activity of tryptophan peroxidase-oxidase (TPO) of rat liver can be augmented *in vivo* by administration of large doses of the substrate, tryptophan, and to a lesser extent by injection of certain other amino acids, epinephrine, or histamine (1). The increase after tryptophan was considered to be an adaptive response, while the effect of non-specific compounds was attributed to pituitary-adrenal stimulation, since adrenalectomy inhibited the response to non-substrate compounds but not to tryptophan.

It was of interest to study the effects of total body x-irradiation on TPO of rat liver

for several reasons. The conversion of tryptophan to formylkynurenine is a coupled two-step oxidation(2), the first step requiring peroxide and the second generating peroxide (which is used with high efficiency in the first step). Secondly an active catalase is necessary to the system to prevent accumulation of inhibitory concentrations of peroxide. Furthermore, free sulfhydryl groups seem to be necessary as inferred from inhibition of the reaction by arsenite. A final consideration was the role of the adrenals in response to irradiation (3), which raised the question whether any changes in activity of TPO produced by x-ray exposure were due directly to irradiation or were attributable to hormonal effects.

Methods. Most of the experiments utilized female Sprague-Dawley or Holtzman rats, 3 months old, weighing between 160 and 210 g; the 2 strains were identical in their response. Hypophysectomized Sprague-Dawley female rats were obtained commercially. Other operative procedures were carried out in the laboratory. Exposure to x-rays was at the rate of 225 r per minute from a machine operated at 250 kv, 15 ma, 0.5 mm Cu and 3.0 mm bakelite filters, 27 cm distance. A dose of 1000 r was used in most experiments. The method of assay for TPO was based on the formation of kynurenine measured spectrophotometrically at 360 mμ after zinc acetate-NaOH deproteinization of a reaction mixture containing homogenized liver, phosphate buffer, and tryptophan incubated at 38°C for 60 minutes(1). Under the conditions employed, kynurenine formation was linear in respect to time for 4 hours, and in respect to tissue concentration to at least 80 mg liver/ml reaction mixture. Measurement of ultraviolet absorption at other wave lengths indicated only negligible absorption attributable to compounds such as anthranilic acid, formylkynurenine, or kynurenic acid; the absence of other metabolites of tryptophan we further confirmed by paper chromatography(4).

Results. A nearly 2-fold increase in TPO activity was noted 4 hours after exposure of the rat to 1000 r. The activity declines gradually, reaching the normal range about 48 to 72 hours after exposure. Studies with varying amounts of radiation showed that the phenomenon was dose-dependent to at least 5000 r, although a linear relationship was not obtained. A significant increase was observable after doses as low as 200 r. Male rats responded as did females. Activation of TPO was also noted after x-irradiation of mice, but not of guinea pigs.

Incubation of mixed homogenates from livers of normal and irradiated rats showed in every case only single summation of activities, an indication that radiation neither destroyed an inhibitor nor created an activator of the system. Measurements of catalase and pyrogallol peroxidase showed no changes in the activities of these enzymes during the first 24 hours after irradiation.

Like the increase caused by epinephrine, histamine, etc.(1), the radiation-induced increase was inhibited by adrenalectomy (Table I). Animals maintained on either salt or cortical extract (Wilson; 0.2 ml/day) failed to show the characteristic response after exposure to 1000 r. Adrenal demedullation decreased the response somewhat but did not abolish it. Thyroidectomized rats did not differ from normals in respect to the activity of TPO after radiation.

Hypophysectomy led to variable results, depending upon the time elapsing between operation and sacrifice. Seven or 10 days after hypophysectomy the increase after x-ray was appreciable, although less than that seen in unoperated animals. The response of TPO became progressively less as the time intervals increased, due probably to atrophy of the adrenal cortex, since maintenance of hypophysectomized rats on 2 units/day of ACTH permitted a considerable restoration of the response in animals irradiated 14 days after operation. In no case, however, was the increase as marked in hypophysectomized rats as in the unoperated animals. A single injection of ACTH alone, even as much as 5 units, failed to produce a consistent increase in activity 4 to 6 hours after injection.

Attempts to inhibit the action of x-ray on TPO by administration of various drugs were not successful. Prior intraperitoneal or intravenous injection of large doses of atropine, physostigmine, tetraethylammonium chloride, procaine, cocaine, diphenhydramine, ephedrine, ascorbic acid, or cysteine produced *per se* a 200 to 300% increase in activity which

TABLE I. Effects of Total Body X-Irradiation and of Injection of Compound F and Tryptophan on Rat Liver Tryptophan Peroxidase.

Rats	Days after operation	Control			4 hr after 1000 r			4 hr after 5 mg/kg Compound F			2 g/kg dl-tryptophan					
											4 hr			6 hr		
		No. rats	Avg*	σ	No. rats	Avg	σ	No. rats	Avg	σ	No. rats	Avg	σ	No. rats	Avg	σ
Normal	—	48	7.7	1.5	16	13.9	2.4	6	15.5	3.4	5	66	9	9	121	18
Adrenalectomized	7- 9	20	7.4	1.9	11	7.0	1.3	4	17.1	2.4	4	60	13	Fatal within 6 hr		
Adrenal demedullated	7-14	7	8.6	1.2	6	10.1	.8	—	—	—				—	—	—
	21-28	13	8.1	1.5	13	11.4	1.1	4	12.1	2.3				7	74	25
Thyroidectomized	35	4	6.8	1.4	4	14.1	1.2	4	13.7	.5				4	81	14
Hypophysectomized	7	4	6.8	.1	4	9.6	.2									
	10	4	7.0	.3	4	9.1	.4									
	14	8	5.4	1.2	8	6.6	1.3	4	23.0	5.3				4	25.2	12.4
	28	4	6.3	.5	4	5.8	1.0									
Hypophysectomized + ACTH	14	4	6.8	.6	4	9.0	1.0	3	24.0	2.9				3	34.5	3.1

* Micromoles kynurenine formed/hr/g dry wt.

effectively masked the effect of radiation on TPO. Similarly, section of the spinal cord at C_6 resulted, after 24 hours, in a 5-fold increase in unexposed animals.

Intramuscular injection of 5 mg/kg of 11-hydroxy-17-corticosterone acetate (Compound F) in saline suspension was found to double the activity of TPO within 4 hours. Smaller amounts were proportionately less effective. This action of Compound F was not appreciably diminished by removal of the adrenal or thyroid, and was enhanced after hypophysectomy (Table I). Desoxycorticosterone acetate and tetrahydrocortisone had no stimulatory effect in adrenalectomized rats. Cortisone, on the other hand, acted like Compound F but was about ½ as effective. It may be pointed out further that neither Compound F nor x-ray exposure effected an increase in liver TPO activity of guinea pigs. The mechanism by which the corticosteroids act is as yet obscure; *in vitro* studies with rat liver slices incubated with Compound F and then homogenized and assayed for TPO activity have been inconclusive.

The 15-fold increase in activity of TPO resulting from injection of 2 g/kg of DL-tryptophan in normal rats (Table I) could be produced in irradiated animals until 4 days after exposure when the rats were manifestly moribund. In confirmation of Knox's findings (1), we found only a slight decrease in "adaptation" to tryptophan in adrenalectomized rats; we have also confirmed the high toxicity of tryptophan to adrenalectomized rats. Adrenal demedullation and thyroidectomy also failed to affect the response of TPO to tryptophan. In hypophysectomized rats, however, the injection of tryptophan produced much less effect than in normal rats (Table I).

Discussion. It is apparent that the increase in activity of the tryptophan peroxidase-oxidase system of rat liver observed after exposure to x-ray is not a direct consequence of radiation of the liver, but rather is secondary to an effect on the adrenals, since the increase does not occur in rats exposed to x-rays after adrenalectomy. Radiation is thus a nonspecific activator of the TPO system, resembling histamine, epinephrine, histidine, etc. (1). The proposed pituitary-adrenal mech-

anism of action of these non-specific activators (1) seems, however, to be open to question.

Summary. Total-body x-irradiation produces within a few hours a dose-dependent increase in the tryptophan peroxidase-oxidase system of rat liver. The increase does not occur in adrenalectomized rats, and hence cannot be construed as a direct effect of x-irradiation.

1. Knox, W. E., *Brit. J. Exp. Path.*, 1951, v32, 462.
2. Knox, W. E., and Mehler, A. H., *J. Biol. Chem.*, 1950, v187, 419.
3. Leblond, C. P., and Segal, G., *Am. J. Roentgenol.*, 1942, v47, 302.
4. Mason, M., and Berg, C. P., *J. Biol. Chem.*, 1951, v188, 783.

Received November 30, 1953. P.S.E.B.M., 1954, v85.

Isolation and Chemical Composition of Complement-Fixing Antigens From Leptospires. (20776)

MORRIS D. SCHNEIDER. (Introduced by R. H. Yager.)
From the Veterinary Division, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D.C.

The purpose of this report is to present a comprehensive account of a fractionation procedure applied to the leptospiral cell and to show the comparative yield of the complement-fixing cell fractions from selected serotypes of leptospires. Fraction 1, a complement-fixing substance, has been isolated and its chemical and serological properties described(1-3).

The process of fractionation of the leptospiral cell has been extended to include the cellular debris remaining after extraction of Fraction 1. The cell residue served as the starting material from which were isolated three serologically active fractions.

Methods. Cultures of leptospires. Three serotypes of leptospires were employed, namely. *Leptospira icterohemorrhagiae* AB, *L. canicola* and *L. bataviae*. Leptospires were propagated and harvested as previously described(1,3). *Isolation of complement-fixing antigens.* The protocol for fractionation of the leptospiral cell is outlined in Fig. 1. Washed viable organisms were disrupted as previously described(1,3). After shaking with one-half volume 4:1 chloroform-n-amyl alcohol, the mixture was centrifuged at 4000 rpm for 20 minutes, at 0°C. The preparation separated into (A) an aqueous phase, (B) an interfacial cell-protein gel or chloroform "cake" and (C) a chloroform layer. The aqueous phase yielded, on further processing, a genus-specific antigen previously described (1-3). The cell protein debris (B) was separated from the chloroform layer, suspended in and thoroughly dialyzed against 0.15 M NaCl solution and disintegrated by sonic vibration. The final volume of this preparation was 1 to 2% of the original culture volume. The crude cell-protein antigen was fractionated as follows: The crude material was precipitated from its aqueous suspension by the addition of one-half volumes of each of 0.2 N H_2SO_4 and freshly prepared 2% sodium tungstate solution. A heavy flocculent precipitate appeared soon after the addition of tungstate solution. After 30 minutes, the acidified mixture (pH about 2.0) was centrifuged at low speed, in the cold, and yielded: 1) A soluble supernatant, which on subsequent dialysis against 0.15 M NaCl solution and pervaporation yielded a non-dialyzable, complement-fixing substance, identified as Fraction 4; 2) An insoluble sedimentable portion which was then suspended in water to one-half of the volume of the original crude suspension. Three volumes of absolute ethanol were added, the mixture shaken and held at about 0°C for 24 to 48 hours. After low speed centrifugation, the latter mixture separated into: a) A clear to slightly opalescent alcoholic phase which, after thorough dialysis against distilled water and pervaporation, yielded a non-dialyzable water-insoluble material designated Fraction