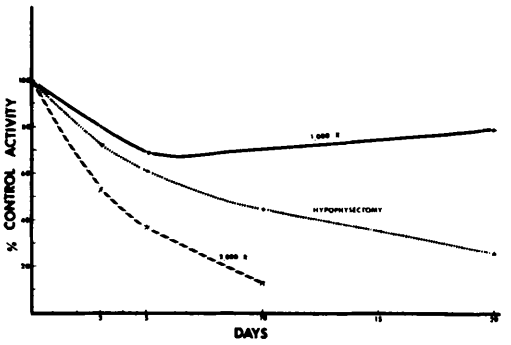

FIG. 3. The response of the hexobarbital metabolising enzyme system in adult male rat liver to a) 1,000 R head x-irradiation, b) 2,000 R head x-irradiation and d) hypophysectomy are compared.

has been reported to suppress the normal developmental increase of enzyme activity seen in male rats(8). It is shown in the present studies (Fig. 3) that hypophysectomy in adult rats (65 days old) also results in an inhibition of the hepatic enzyme system. Although the mechanisms underlying the abscopal effects of head irradiation on liver enzyme activity cannot be outlined yet, collateral evidence suggests that radiation impairment of the hypophyseal regulation may be responsible for the distant effects observed. It is also apparent from our results that the central nervous system exerts a regulatory role not only in the development but also in the maintenance of an optimal level of enzyme activity in liver.

Summary. Exposure to x-irradiation *in utero* or during the early postnatal life (total body or head alone) produced a suppression of the development of hexobarbital metabolising enzyme system in liver. Abscopal inhibition of the hepatic enzyme system after x-irradiation was also noted in adult male rats, but with higher radiation doses, suggesting that the central nervous system plays a regulatory role not only in the development but also in the maintenance of optimal level of enzyme activity in liver.

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1. Gillette, J. R., Progress in Drug Research, edit. by Jucker, E., Birkhauser, Basel, 1963, v6, 11.
2. Conney, A. H., Burns, J. J., Adv. in Pharmacol., 1962, v1, 31.
3. Hietbrink, B. E., DuBois, K. P., Rad. Res., 1964, v22, 598.
4. Nair, V., Zeitlin, S., Rad. Res., 1967, v31, 609.
5. Yam, K., DuBois, K. P., USAF Rad. Lab. Quart. Progr. Rep. No. 56, 1965, 36.
6. Cooper, J. R., Brodie, B. B., J. Pharmacol., 1955, v114, 409.
7. Quinn, G. P., Axelrod, J., Brodie, B. B., Biochem. Pharmacol., 1958, v1, 152.
8. Yam, K., DuBois, K. P., Rad. Res., 1967, v31, 315.

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Serological Specificity of Types A and B Botulinal Toxins and Antitoxins. (32588)

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Types A and B botulinal toxins and antitoxins appear to be quite specific as determined by animal neutralization tests(1). Multiple precipitin bands and cross-reactions have been observed, however, in gel-diffusion tests. Lamanna and Lowenthal(2) showed that type A crystalline botulinal toxin formed two precipitin bands with type A antitoxin in the Oudin gel-diffusion test and one band with type B antitoxin. They demonstrated that one of the homologous bands and the cross-reacting band were attributable to the hemagglutinin in type A toxin and its antibody present in the homologous and heterologous antitoxins. Gendon(3) demonstrated numerous bands between crude type A toxin

and antitoxin in the Oakley double-diffusion tube test. Because of the lack of purity of the reagents with which he worked, some of these bands were probably produced by substances other than toxin and antitoxin.

Cross-reactions were observed in a study of types A and B botulinal toxins, employing the passive hemagglutination and bentonite flocculation tests(4). Type B antitoxin cross-reacted with A toxoid in passive hemagglutinations, and reciprocal cross-reactions were observed in bentonite flocculations. Titration of A and B antitoxins in the presence of the heterologous toxoids eliminated these cross-reactions without affecting the homologous systems. Toxins from only one strain each of A and B toxin-producing organisms were used in these studies. This report presents data on several strains each of types A and B *Clostridium botulinum* organisms in the passive hemagglutination and gel-diffusion tests. Rabbit antitoxins, equine antitoxins from the National Communicable Disease Center (NCDC) and equine International Standard antitoxins were used. The purpose of this study was to determine the serological relationships of types A and B botulinal toxins and antitoxins obtained from different organisms and sources. Data of this nature are pertinent to the development of an *in vitro* assay procedure for botulinal toxins.

Materials and methods. Toxoids. Purified botulinal toxoids types A and B, in approximately 0.5% formalin, were obtained from Dr. George Wright, U.S. Army Biological Laboratories. Toxoids were exhaustively dialyzed against phosphate-buffered saline (PBS), pH 7.2(5), before use in serological tests.

Antitoxins. Types A and B equine antitoxins were obtained from the National Communicable Disease Center, Atlanta, Georgia. Equine International Standards for types A and B antitoxins were obtained from the Statens Seruminstitut, World Health Organization, Copenhagen, Denmark. The International antitoxins were received in the dried state and were dissolved in 0.25% bovine serum albumin in PBS to a concentration of 100 units per ml and stored in the frozen state prior to use. Hyperimmune rabbit antitoxins types A and B were produced as previously described(4).

Toxic cultures. Stock cultures were obtained orginally from NCDC and the U. S. Army Biological Laboratories. They were grown for toxin production in a medium developed by Dr. Francis Crisley, Milk and Food Research. The medium, designated medium B, was prepared by adding 50.0 grams oxoid peptone* and 5 grams "Bacto" yeast extract to 1,000 ml distilled water. The pH was adjusted to 7.0-7.2, and the medium was autoclaved. Before seeding with a culture, dextrose was added to a final concentration of 0.5%. The medium was heavily inoculated with the culture, which had been primed in thioglycollate broth. The culture was incubated for 4 days at 35°C and centrifuged at 5,000 rpm for 30 min in a high speed centrifuge (Sorvall RC-2). The supernatant fluid was assayed for toxin as previously described(4) and employed in hemagglutination inhibition and gel-diffusion tests.

Hemagglutinations (HA). HA with toxoids coupled to formalinized sheep red blood cells (SRBC) by bis-diazotized benzidine were performed as previously described(4).

Hemagglutination inhibitions (HI). HI were performed by adding 0.25 ml of various dilutions of supernatant culture fluids to equal volumes of 2-fold serially diluted antitoxin. The dilution of antitoxin was based on a final volume of 0.5 ml. Following incubation at room temperature for 20 min, hemagglutinations were carried out. The control consisted of serially diluted antiserum titrated in the presence of culture medium. An 8-fold or greater reduction in titer over that of the control was considered as specific inhibition.

Gel-diffusions. Micro-Ouchterlony gel-diffusions were performed as previously described(6).

Results. Data on comparative hemagglutinations with A and B botulinal toxoids and the 3 sources of corresponding antitoxins are presented in Table I. In all systems, the homologous reactions were stronger than the heterologous. The degree of comparative cross-

* Mention of commercial products throughout has been made only to identify items that cannot be completely described and is not to be construed as endorsement by the Public Health Service.

TABLE I. Passive Hemagglutination with Types A and B Botulinal Antitoxins.

Toxoid	Antitoxin source	Antitoxin* (reciprocal of dilution) A	B
A	Rabbit	67,000,000	524,000
B		65,500	8,400,000
A	NCDC, equine	25,600 (0.0004)	3,200 (0.003)
B		<100 (>0.1)	51,200 (0.0002)
A	International Standard, equine	1,640,000 (0.00006)	102,400 (0.001)
B		3,200 (0.03)	820,000 (0.00012)

* Values in parentheses indicate the units of antitoxin per ml in the end point tubes. The neutralizing capacities of the rabbit antitoxins were not quantitated.

reactivity is illustrated in the determination of the ratio of the homologous to heterologous titers (Table II). In all systems, the ratio of homologous to heterologous reaction was much greater than 1, demonstrating the specificity of the HA. The degree of cross-reactivity was similar for the different antitoxin systems. The cross-reactivity of A toxoid-SRBC and B antitoxin was much greater than the reciprocal cross-reaction. A similar pattern was also noted with the antitoxins used in a previous study(4).

TABLE II. Ratio of Homologous to Heterologous Hemagglutination (HA) Titers with Types A and B Botulinal Antitoxins.

Antitoxin, source	Toxoid	HA titer, homologous / heterologous
A, Rabbit	A/B	1024
B, "	B/A	16
A, NCDC	A/B	>256
B, "	B/A	16
A, International	A/B	512
B, "	B/A	8

Hemagglutination inhibition data with types A and B botulinal cultures and the 3 sources of antitoxins are presented in Table III. The patterns of inhibition for the different antitoxins were quite similar. 3 type A cultures (Hall, CAA, 33), for example, specifically inhibited hemagglutinations with A antitoxins, although the results with CAA and rabbit anti B were equivocal. Cultures 297 and 1156, although of considerable toxicity, failed to inhibit hemagglutination with the 3 A antitoxins. These inhibitions were repeated numerous times with essentially the same results. All B cultures with sufficient toxicity (Beans, 770, 1541) gave significant and specific inhibitions with B antitoxins. Culture 41, which was of extremely low toxicity, did not inhibit either the A or B systems. This is in agreement with the limits of sensitivity of inhibitions that were established previously(4). The possible significance of these data will be discussed.

Specificities and serological relationships among types A and B toxins and antitoxins were further studied by micro-Ouchterlony

TABLE III. Hemagglutination Inhibitions with Botulinal Cultures.

Culture, type	LD_{60}/0.25 ml	Fold reduction in titer*: Rabbit antitoxin A	Rabbit antitoxin B	NCDC equine antitoxin A	NCDC equine antitoxin B	International equine antitoxin A	International equine antitoxin B
Hall, A†	14,000	>512	2	>512	2	>512	2
CAA, A	6,000	64–128	4–8	32	2–4	32–64	2–4
33, A	6,000	32–64	4	16–32	2	64–128	0–4
297, A	6,000	2	4	0–2	2	0–2	0–4
1156, A	535	0	2–4	0	0–2	0	0
Beans, B†	6,000	0	>512	0	>512	0	>512
770, B	10,000	2–4	128–512	2	128	2–4	128–512
1541, B	790	0	256	0	256	0	64
41, B	6	2–4	2–4	2–4	0–2	0–4	0–2

* Eight-fold or greater reduction in titer is considered as specific inhibition.
† Strains employed in previous hemagglutinations(4).

FIG. 1. Diagrammatic representation of gel-diffusion reactions of types A and B rabbit antitoxins and their corresponding toxoids. A, rabbit A antitoxin; B, rabbit B antitoxin; 1, 2, 3, B toxoid; 4, 5, 6, A toxoid.

gel-diffusions. Fig. 1 presents gel-diffusion patterns obtained with rabbit types A and B antitoxins and A and B toxoids. The toxoids were from the same lot used in HA. Type A antitoxin produced 2 lines with its homologous toxoid, the innermost of which showed identity with B toxoid. Type B antitoxin, on the other hand, produced 3 lines with its homologous toxoid. The middle line showed identity with A toxoid. The innermost line was quite weak and could not be reproduced with consistency. The line of identity between the heterologous systems is probably due to the hemagglutinin referred to by Lamanna and Lowenthal(2). NCDC and International antitoxins gave reactions similar to those of rabbit antitoxins, although they were weaker. The innermost line with rabbit type B antitoxin could not be produced with the NCDC and International antitoxins.

Supernatant fluids from the type A cultures employed in the inhibitions in Table III were also examined by gel-diffusion against rabbit type A antitoxin (Fig. 2). The A toxoid as well as strains CAA and 33 gave identical reactions. No lines were observed with cultures 297 and 1156 even after numerous ratios of culture and antitoxin were tested. The precipitin reactions, then, are in agreement with the HI data for these cultures. Although not shown, the Hall strain produced results identical to A toxoid.

Rabbit type B antitoxin and the B cultures used in HI were also examined by gel-diffusion (Fig. 3). Culture 770 gave 3 bands, a reaction identical with that of B toxoid. Although not shown, Bean strain also gave this type of reaction. Culture 1541 produced only 1 band, the innermost, with B antitoxin. It is possible that this is the system involved in HA and HI, since 1541 was an effective inhibitor of HA. Culture 41, which was virtually non-toxic and non-inhibitory, produced 1 precipitin band, which corresponded to the hemagglutinin-anti-hemagglutinin.

Discussion. In general, the behavior of the rabbit, NCDC equine, and International

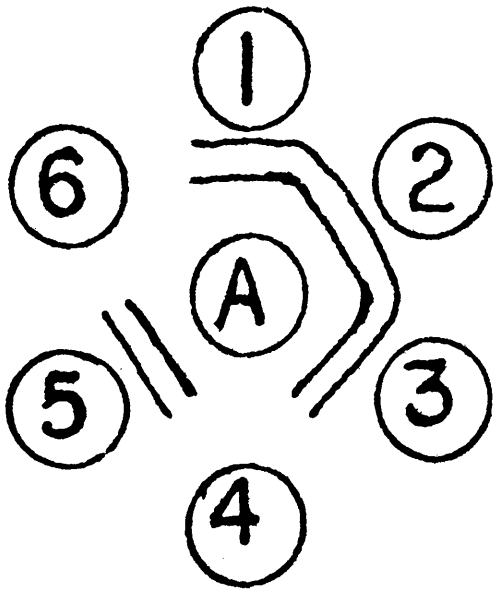

FIG. 2. Diagrammatic representation of gel-diffusion reactions of type A rabbit antitoxin and type A botulinal culture fluids. A, rabbit A antitoxin; 1, culture CAA; 2, 5, A toxoid; 3, culture 33; 4, culture 1156; 6, culture 297.

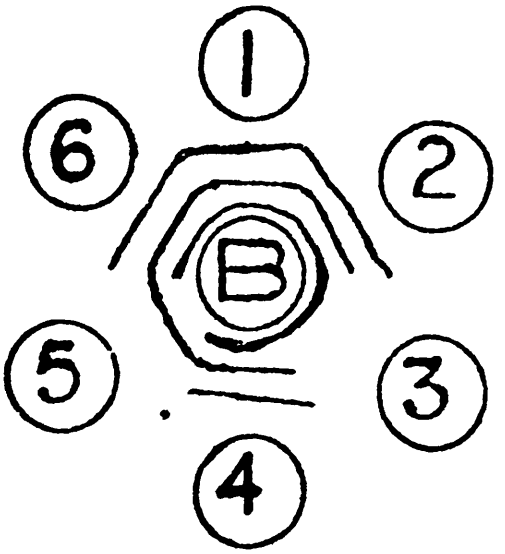

FIG. 3. Diagrammatic representation of gel-diffusion reactions of type B rabbit antitoxin and type B botulinal culture fluids. B, rabbit B antitoxin; 1, culture 770; 2, 4, 6, B toxoid; 3, culture 1541; 5, culture 41.

Standard equine antitoxins was quite similar in HA, HI, and gel-diffusions. The cross-reactions observed in HA and gel-diffusions were probably due to the hemagglutinins present in A and B toxins, which have been shown to be antigenically similar(2). The specificity of the HA reactions suggested that the hemagglutinin was not the most significant antigenic component of the toxin molecule. The patterns of HI and gel-diffusions with type A toxins indicated that the *in vitro* serological specificity of A toxin was not associated with neutralizing antibodies. Type A cultures 297 and 1156, for example, did not inhibit HA and failed to precipitate with type A antitoxins in gel-diffusions. This cannot be attributed to low toxicity of the cultures, since 297 was as toxic as cultures CAA and 33, both of which inhibited HA and formed 2 bands in gel-diffusions with A antitoxins.

The data on HI with B toxic cultures did not eliminate the possibility that neutralizing antibodies played a significant role in the HA reaction, since HI was obtained with all of the moderately to highly toxic cultures, but not with 41, which was of extremely low toxicity. Type B cultures Bean and 770, and B toxoid produced 3 lines of identity against B antitoxin. Culture 41 formed only 1 line, which corresponded to the hemagglutinin-antihemagglutinin shown to be of no significance in the HI reaction. Culture 1541, which was highly effective in the HI reaction, formed only 1 line of precipitate in the gel-diffusion test. This was the innermost line which showed identity with the innermost line of the other cultures that were effective in HI. It is possible that the innermost line represented the significant system in HA. Data presented here did not indicate its definite relationship with the toxic moiety of the B toxin and the neutralizing antibody.

Supernatant fluids from cultures of a non-toxic variant of the Beans strain of *C. botulinum* inhibited B toxoid HA (H. M. Johnson, unpublished data), when reagents employed in an earlier study(4) were used. Some recent cultures of this non-toxic variant were found to have no effect on the toxoids and antitoxins that were used in obtaining the data reported here. Unfortunately, the reagents employed in the earlier study are no longer available. Studies are continuing with other non-toxic variants in an attempt to explain this phenomenon.

Botulinal toxins purified in the manner as described for the toxoids used in the present study consist of toxic and non-toxic fractions (7). Recent studies have been conducted to isolate the toxic fractions. Gerwing *et al*(8,9), employing chromatographic techniques, isolated toxic moieties of molecular weights approximating 10,000 from types A and B, *C. botulinum* cultures. Boroff *et al*(10) employed chromatographic procedures for the fractionation of crystalline type A toxin into a highly toxic moiety of molecular weight 128,000 and a feebly toxic moiety that had a sedimentation coefficient of S_{20}, W = 13. It would be of considerable interest to observe the *in vitro* serological behavior of these toxic substances. Such studies might result in the improvement in correlating data obtained by *in vitro* and *in vivo* procedures.

Summary. Rabbit, National Communicable Disease Center (NCDC) equine, and International Standard antitoxins to types A and B botulinal toxins were compared by passive hemagglutination (HA), hemagglutination in-

hibition (HI), and gel-diffusion. The HA reactions in general were quite specific, although cross-reactions were observed. The extent of cross-reaction was greater with B antitoxins and A botulinal toxoid-sensitized red blood cells. The different antitoxins behaved similarly in HI and gel-diffusions, though the reactions of the NCDC and International antitoxins were weaker than those of rabbit antitoxins in gel-diffusions. Hemagglutination inhibition and gel-diffusion reactions with toxic cultures suggested that the *in vitro* specificity of the A toxin-antitoxin did not involve neutralizing antibodies. Hemagglutination inhibitions and gel-diffusions with B toxins did not resolve the question of the involvement of neutralizing antibodies in *in vitro* specificity, since fairly toxic cultures inhibited HA and formed bands believed to be associated with type specificity, whereas a virtually non-toxic B culture neither inhibited HA nor formed a line of identity with the type specific systems in gel-diffusion.

1. Dolmen, C. E., Botulism as a World Health Problem, U. S. Public Health Service Publ. No. 999-FP-1, 1964, p5.

2. Lamanna, C., Lowenthal, J. P., J. Bacteriol., 1951, v61, 751.

3. Gendon, Iu Z., J. Microbiol. Epidemiol. and Immunobiol., 1958, v29, 693.

4. Johnson, H. M., Brenner, K., Hall, H. E., J. Bacteriol., 1966, v97, 791.

5. Stavitsky, A. B., J. Immun., 1954, v72, 360.

6. Crowle, A. J., J. Lab. Clin. Med., 1958, v52, 784.

7. Schantz, E. J., Purification and Characterization of *C. botulinum* Toxins, U.S. Public Health Service Publ. No. 999-FP-1, 1964, p91.

8. Gerwing, J., Dolman, C. E., Bains, H. S., J. Bacteriol., 1965, v89, 1383.

9. Gerwing, J., Dolman, C. E., Kason, D. V., Tremaine, J. H., ibid., 1966, v91, 484.

10. Boroff, D. A., Townsend, R., Fleck, U., Das Gupta, B. R., J. Biol. Chem., 1966, v241, 5165.

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Thyroid State and Vascular Reactivity in Rats.* (32589)

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The assumption that changing the thyroid state alters the responsiveness of the organism to catecholamines has been prevalent for many years(1). Arterial blood pressure studies have suggested that the vascular responses to catecholamines, and perhaps to other agents as well, are enhanced by hyperthyroidism and diminished in hypothyroidism(1,2). However, recent reports indicate that the responsiveness of myocardial and vascular smooth muscle to catecholamines may not always be augmented by hyperthyroidism in dogs(3,4).

The following studies describe the effects of altered thyroid state on the sensitivity of rats to the vasoconstrictor actions of norepinephrine and angiotensin. Changes in vascular resistance, independent of changes in systemic arterial blood pressure, were estimated using an auto-perfused hindquarter preparation.

Materials and methods. Male Sprague-Dawley rats (initially weighing 200 to 250 g) were used in this study. Hypothyroidism was produced in 9 rats by radiothyroidectomy (850 μc NaI^{131} I.P.) at least 2 weeks prior to the experiments. These rats were fed a Remington iodine deficient diet for 1 week prior to radiothyroidectomy and Purina Lab Chow thereafter. Rats of other groups were similarly fed the iodine deficient and then the standard diet. At autopsy, no functional thyroid tissue could be detected histologically in these animals. Hyperthyroidism was in-

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