injected dose per organ. Of the tissues examined, the only instance of a major deviation from control values was in the femurs of animals injected with P^{32}. In this case, the P^{32} content of the femurs of animals sacrificed 30 minutes after injection was only about half that of the controls. The difference was reflected in generally higher values in some of the soft tissues. After 1 hour this difference was reduced considerably and disappeared entirely in mice sacrificed after 1 day. The difference in femur retention of P^{32} at 30 minutes is considered especially significant since the reproducibility of bone values from animal to animal is good. The data further indicate that in the case of Sr^{90} BAL has no effect on either the rate of uptake, distribution, or total retention.

These results indicate that although the intramuscular administration of BAL in mice receiving P^{32} intraperitoneally results in a reduced rate of deposition of the isotope in bone, the differences are obliterated within 24 hours. No differences in the total retention of P^{32}, as compared with controls not treated with BAL, were observed. It would appear, therefore, that the use of BAL as an agent to accelerate elimination of either P^{32} or Sr^{90} from the animal body is not practicable, at least under the conditions of this experiment.

Summary. The effectiveness of 2,3-dimercaptopropanol (British anti-lewisite) in increasing the elimination of injected P^{32} and Sr^{90} in mice was investigated. It is concluded that BAL is essentially without effect although, in the case of P^{32}, the rate of uptake in bone appears to be reduced during the first hour. No such differences could be demonstrated after 24 hours.

Received June 22, 1951. P.S.E.B.M., 1951, v77.

Anaphylactic Hypersensitivity Induced in Rabbits to Foreign Proteins in Freund's Adjuvant.* (18897)

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(Introduced by G. L. Duff.)

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The method of enhancing the sensitivity of animals to foreign proteins by the incorporation of the antigen in Freund's adjuvant containing *M. tuberculosis* is well known. Although the originators of the method(1) did not believe that the sensitivity to the proteins was more than quantitatively different from that induced by the proteins used alone, the impression has persisted(2,3) that the state of hypersensitivity induced by the foreign proteins in Freund's adjuvant is of the tuberculin type. It may be that this impression dates from the work preceding the description of the adjuvant(4-6), in which it was held that, in tuberculous guinea pigs, the state of hypersensitivity induced to foreign proteins was of the tuberculin type. However, this interpretation was by no means established as fact(7).

The possibility that one might alter the type of hypersensitive reaction to a given foreign protein appeared to be of considerable

* Assisted by a Grant-in-aid from the National Research Council of Canada.

† Post-doctoral Fellow of the Life Insurance Medical Research Fund.

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importance in considering the relationship between the state of hypersensitivity and the cardiac valvulitis, necrotizing arteritis and glomerulonephritis that can be induced in rabbits by massive injections of horse serum (8-11) or bovine gamma globulin(12-14). Short term experiments employing 2 injections of horse serum or bovine gamma globulin incorporated in Freund's adjuvant containing *M. tuberculosis*(15) indicated that lesions of the heart, arteries and kidneys could be produced in the animals and that the skin sensitivity that developed was of the usual Arthus or anaphylactic type. It was considered desirable to make further observations on animals treated for a longer period of time and to extend them to include observations on the skin reactions to tuberculin and on the possibility of passive transfer of the sensitivity by serum.

Materials and methods. Two groups each consisting of 7 albino rabbits were given intramuscular doses of 0.1 g of lyophilized horse serum protein or bovine gamma globulin in Freund's adjuvant at weekly intervals for 18 weeks. Two further injections were given 8, 16 and 17 weeks after the last injection. Thus the animals received 21 injections in all over a period of 35 weeks. One week after the last injection the 7 surviving animals were bled and skin tested to graded dilutions of bovine gamma globulin or horse serum. The same animals were skin tested with varying amounts of purified protein derivatives (P.P.D.). Some of the animals have died since these tests were made and others are being kept alive to observe the effect of the injected substances over longer periods of time.

The Freund's adjuvant used in these experiments contained a final concentration of 0.6 g of dried horse serum per 5 cc of the mixture or 1.0 g of bovine gamma globulin per 4 cc. These antigens were first suspended in 2 parts of saline in a mortar. This viscid mixture was then combined with 1 part of Falba‡ in the mortar. Following this, 2 parts of Bayol F¶ containing 2.5 mg of killed and dried *M. tuberculosis* per cc, were added to the previous constituents and the whole emulsified in a Waring Blendor. Each injection consisted of 0.4 cc of the adjuvant-globulin mixture and 0.8 cc of the adjuvant-horse serum mixture.

Intradermal tests were made to saline and graded dilutions of horse serum or 10% bovine gamma globulin in the following dilutions: 1/1, 1/10, 1/100, 1/1000, and to P.P.D. in quantities of .0025 mg, .005 mg, and .01 mg as well as to the diluent used to dissolve the P.P.D. The reactions were read at one hour, 24, 48, 72, 96 and 160 hours by measuring the maximum and minimum diameters of the resulting oedema, redness, haemorrhage or necrosis. From these the average diameters of the phases of the reactions were calculated for each individual reading and the results tabulated. Since the results seen in the individual animals did not differ qualitatively from one another whether they received horse serum or bovine gamma globulin, all the average diameters were combined, tabulated in Table I and represented graphically in Fig. 1. The height of the oedema was roughly proportional to its average diameter but the former is so difficult to measure accurately that the numerical data concerning height were not considered suitable for analysis.

Serum from the sensitized rabbits was injected intraperitoneally into guinea pigs in quantities of 2.0-2.5 cc. After 24-30 hours an intravenous shocking dose of 0.2 cc of the appropriate antigen was given.

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¶ Obtained through the courtesy of Stanco, N. Y.

TABLE I. Skin Tests. Average diameter in mm. Avg of all animals tested(7).

Antigen and dilution		Hr after test dose 1	24	48	72	96	160
Saline	0	7.21	0	0	0	0	0
Serum 1/1	R*	0	34.9	35.3	32.8	27.2	19.5
	O	14.02	34.9	35.3	35.1	34.2	24.6
	H	0	8.57	6.6	3.2	.3	0
	N	0	13.07	12.1	10.7	11.9	11.5
1/10	R	0	25.14	18.2	14.6	10.5	7.2
	O	10.62	25.14	19.3	13.9	15	9.4
	H	0	14.5	2.2	0	0	0
	N	0	8.5	7.7	7.14	6.1	5.1
1/100	R	0	14.78	12.2	9.6	5.4	.85
	O	10.78	14.78	12.2	8.9	6.1	.85
	H	0	6.5	1.1	2.3	.9	0
	N	0	.85	1.07	.14	0	.3
1/1000	R	0	6.28	4	1.1	0	0
	O	9	3.2	.8	1.1	0	0
	H	0	0	0	0	0	0
	N	0	0	0	0	0	0
P.P.D. diluent	R	0	2.14	.6	0	0	0
	O	7.28	.72	0	0	0	0
	H	0	0	0	0	0	0
	N	0	0	0	0	0	0
P.P.D. .0025 mg	R	0	5.2	6.9	7	7.1	7.5
	O	7.9	0	1.8	8.8	5.5	5.3
	H	0	0	0	0	0	0
	N	0	0	0	0	0	0
.005 "	R	0	7.92	10.6	11.8	9.6	8.9
	O	11.35	0	5.8	9	9.6	8.9
	H	0	0	0	0	0	0
	N	0	0	0	0	0	0
.01 "	R	0	8.3	11.6	12.9	13.1	10.5
	O	12.35	0	3.6	9.9	11.5	10.5
	H	0	0	0	0	0	0
	N	0	0	0	0	0	0

* R—redness; O—oedema; H—haemorrhage; N—necrosis.

The precipitin titers of the rabbit sera were determined by finding the optimal ratio(16).

Results. From Table I and Fig. 1 it may be seen that the reactions occurring at one hour to serum are very little different from those obtained with the saline injection and those to P.P.D. differed little from that produced by the diluent. Thus the one hour reading may be considered to be a non-specific one. The slight difference in size of the reactions resulting from the injection of the foreign protein and P.P.D. solutions may be merely a matter of the osmotic pressures of the solutions containing protein preventing the rapid diffusion of the solutions into the tissues. It is also evident from Table I and Fig. 1 that the reactions to serum reached their maximum size in 24 hours and then began to recede. Only with undiluted serum where necrosis of tissue was extensive, did the reactions tend to persist near their original intensity. With the more dilute solutions, a rapid recession occurred. The persistence of the reactions to undiluted serum may therefore be considered to be a result of the marked necrosis of tissue. The fundamental difference between the reactions to the serum and to the P.P.D. may be seen in Table I and in Fig. 1 where it was evident that even in the absence of necrosis of tissue the reactions to P.P.D. tended to be delayed in type and reached their maximum in 72-96 hours and were persistent at almost their maximum intensity until the 7th day. It was therefore concluded that while the tuberculin type of sensitivity did develop in these animals to tuberculin as a result of the injection of whole *M. tuberculosis*

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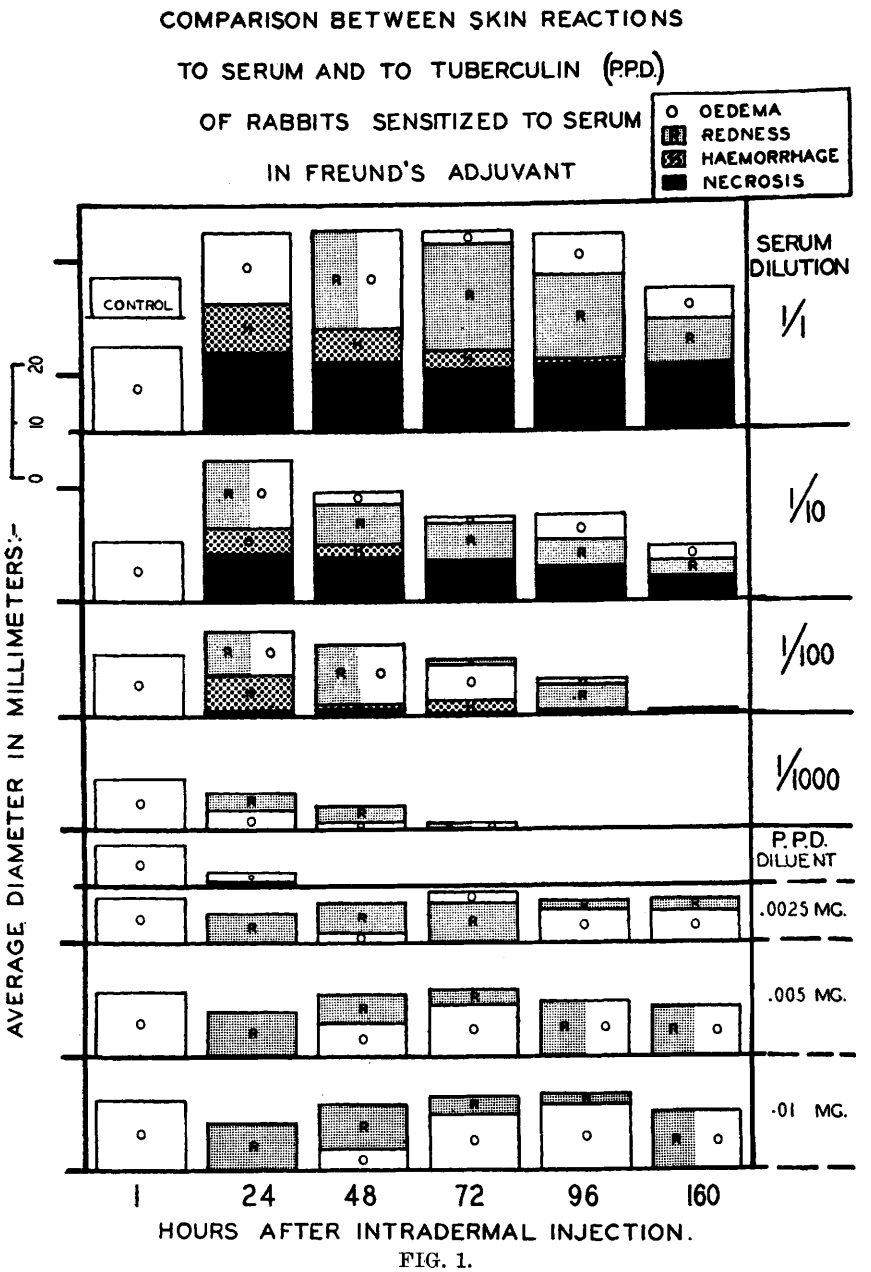

FIG. 1.

as a constituent of the Freund's adjuvant, the type of sensitivity that developed to the serum while incorporated in this vehicle, was of the typical anaphylactic or Arthus type. While this is true of the reactions in rabbits the possibility that the type of skin sensitivity may be altered in guinea pigs by use of the adjuvant cannot be excluded.

Further evidence that the sensitivity to the injected serum was of the anaphylactic type was the demonstration of circulating precipitins in the serum of the treated rabbits shown in Tables II and III. In these tables it is also shown that the sensitivity could be passively transferred to guinea pigs in that the majority of the animals manifested fatal

TABLE II. Passive Transfer of Anaphylactic Sensitivity to Guinea Pigs Using Anti-Bovine Gamma Globulin Rabbit Serum.

Rabbit No.	Optimal ratio	No. of guinea pigs sensitized	Anaphylactic shock			
			Fatal	Severe	Mild	None
I-8	1/128	5	5	0	0	0
I-11	1/64	5	5	0	0	0
I-18	1/128	5	4	0	0	1
I-10	1/64	5	2	1	1	1
H-72	1/128	5	3	0	0	2
P-90	Normal Control Serum	5	0	0	0	5

TABLE III. Passive Transfer of Anaphylactic Sensitivity to Guinea Pigs Using Anti-Horse Rabbit Serum.

Rabbit No.	Optimal ratio	No. of guinea pigs sensitized	Anaphylactic shock		
			Fatal	Severe	None
I-7	1/12	7	5	1	1
I-3	1/16	6	4	0	2
I-17	1/16	6	6	0	0
I-20	1/16	6	5	0	1
H-71	1/16	6	5	0	1
L-22 to L-65	Normal Rabbit Serum	7	0	0	7

or severe anaphylactic shock when challenged with an appropriate intravenous injection of the antigen.

Summary and conclusions. By the use of 3 criteria, namely: the presence of circulating antibodies in the serum; the mode of development of the skin reactions to intradermal injections of antigen, and the passive transfer of anaphylactic sensitivity to guinea pigs; it has been demonstrated that the sensitivity induced in rabbits to foreign proteins while incorporated in Freund's adjuvant is of the usual anaphylactic or foreign protein type. The animals simultaneously develop a tuberculin sensitivity due to the *M. tuberculosis* organisms injected but this in no way alters the quality of the sensitivity developed to the foreign proteins. The persistence of the skin reactions to the higher concentrations of the serum antigens appears to be a reaction to the marked necrosis of tissue that occurs in these highly sensitized animals to the concentrated antigens rather than to any fundamental qualitative difference in the type of reactivity induced in the animals.

Received June 25, 1951. P.S.E.B.M., 1951, v77.

Effects of Oral and Intrasplenic Administration of Cortisone.* (18898)

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It has been found in our laboratories that

* The cortisone acetate suspension was generously supplied by the Merck Corp. through the courtesy of Dr. A. Gibson. We are indebted to Dr. J. J. Schneider, Division of Endocrine and Cancer Research, Jefferson Medical College, for preparing the cortisone hemisuccinate used in this work. The technical assistance of Mrs. M. Sammaritano is gratefully acknowledged.