Immunol., 1957, v79, 80.

5. Ishizaka, T., Ishizaka, K., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 845.

6. Kunkel, H. G., Franklin, E. G., Mueller-Eberhard, H. J., *J. Clin. Invest.*, 1959, v38, 424.

7. Fahey, J. L., Horbett, A. P., *J. Biol. Chem.*, 1959, v234, 2645.

8. Deicher, H. R. G., Holman, H. R., Kunkel, H. G., *Arthr. and Rheumat.*, 1960, v1, 3.

9. Mayer, M. M., Osler, A. G., Bier, O. G., Heidelberger, M., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 66.

10. Kabat, E. A., Mayer, M. M., *Experimental Immunochemistry*, 2nd ed., Charles C Thomas, Springfield, Ill., USA, 1961.

11. Steffen, C., *Klin. Wochenschr.*, 1962, v40, 613.

12. Taranta, A., Franklin, E. C., *Science*, 1961, v134, 1981.

13. Amiraian, K., Leikhim, E. J., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 454.

14. Ishizaka, K., Ishizaka, T., Sugahara, T., *J. Immunol.*, 1962, v88, 690.

15. Taranta, A., personal communication.

16. Spiegelberg, H., personal communication.

Received April 30, 1963. P.S.E.B.M., 1963, v113.

In vivo Incorporation of Iodinated Anti-Rat Brain Globulin and Normal Globulin into Rat Organs. (28441)

CLARA H. WILLIAMS and BEN ROTHFELD
(Introduced by J. L. Rabinowitz)
Psychophysiologic Laboratory and Radioisotope Laboratory, VA Hospital, Perry Point, Md.

The use of antiorgan sera to produce damage to the corresponding organ has been reported frequently: for kidney, Masugi(1) and Baxter(2); pancreas, Armin(3); colon, LeVeen(4); thyroid, Witebsky(5); but not for brain. Kolb and Bolton(6) failed to produce changes in rats after intravenous injections of anti-rat brain serum. Williams(7,8) produced a transient encephalopathy in dogs after intrathecal injections of anti-dog brain serum (on 2 occasions after i.v. injections), and there were deviations from the normal brain histology. When anti-rat brain serum was injected intravenously or intrathecally into rats no such reaction occurred.

Since it was felt that perhaps changes in rat brain after injecting anti-rat brain serum were not sufficiently marked to be observable grossly, labelled anti-brain serum was used to determine whether the antibody was localizing in the brain or not.

The results of studies of intravenous and intrathecal injections of I^{131} and I^{125} labelled anti-rat brain rabbit globulin and normal rabbit globulin into rats sacrificed at varying intervals after injection are reported here.

Materials and methods. Production of antiserum. The antigen used for antiserum production was a saline homogenate prepared by grinding fresh rat brain in saline to make a 10% suspension (10 g per 90 cc saline).

White male rabbits weighing approximately 7 kg were injected intraperitoneally twice a week starting with 1 cc of suspension and increasing to 4 cc until the serum obtained from a trial bleeding gave a high precipitin titer against the supernate from the rat brain homogenate, usually 1-800 to 1-1600. The complement-fixation titer averaged 1-160.

Blood was obtained from several rabbits by cardiac puncture, kept overnight in the refrigerator, the sera were removed, pooled and stored at −14°C until used. *Preparation and labelling of gamma globulin.* Jager and Nickerson's method(9) of precipitation with saturated ammonium sulfate at pH 7 was used to separate the antibody-containing gamma globulin from the rabbit serum. Normal rabbit serum globulin was also prepared. Ten cc of the resulting globulin solution was dialyzed overnight against 1 liter of borate buffer (0.16 M NaCl and 0.20 M H_3BO_3 brought to pH 8 with NaOH). The contents of the dialysis bag were concentrated by suspending the bag in front of a stream of air until the globulin content was 8-10 mg per cc as measured by the Greenberg method.

Iodination of the globulin was performed according to the method of Goodland *et al.* (10) with the modification that the globulin-

TABLE I. Distribution of I^{131} Labelled Anti-Rat Brain Globulin and Normal Globulin in Rat Organs After Intravenous Injections.

Date	No. of rats	Interval	Percent of dose per 100 g rat						
			Blood	Brain	Liver	Kidney	Heart	Spleen	
6/27/62	1	2 hr	12.3	.16	1.81	2.11	1.91	2.79	Normal
	1	" "	2.2	.036	.56	.77	.46	.57	Anti
			5.5	4.5	3.2	2.8	4.2	4.9	Ratio N/A
6/28/62	1	24 "	4.0	.074	.72	.81	.93	.83	Normal
	1	" "	2.2	.038	.45	.70	.52	.57	Anti
			1.8	2.1	1.6	1.5	1.8	1.5	Ratio N/A
6/29/62	1	48 "	6.5	.095	.95	.84	1.37	1.51	Normal
	1	" "	2.9	.048	.41	.60	.60	.87	Anti
			2.2	2.0	2.3	1.4	2.3	1.7	Ratio N/A
7/ 2/62	1	5 days	3.0	.046	.39	.37	.60	.95	Normal
	1	" "	2.0	.034	.37	.38	.40	.54	Anti
			1.5	1.3	1.4	1.0	1.5	1.8	Ratio N/A
7/ 5/62	1	8 "	2.9	.045	.33	.43	.58	.62	Normal
	1	" "	.041	.004	.051	.099	.018	.063	Anti
			70	11	6.5	4.4	3.2	9.9	Ratio N/A

radioiodine solution was dialyzed for two 20 hr periods against 1 liter of fresh 0.85% saline. The globulin content of this solution was measured and the complement-fixation titer determined. This iodinated, dialyzed, anti-rat brain globulin fixed complement up to a 60-fold dilution, and the normal globulin at a one-fold dilution only. (A precipitin titer could not be obtained because of autoprecipitation of the globulin.) In most of the experiments, I^{131} was used to label both the antiserum and normal serum globulin. In one experiment the normal serum globulin was labelled with I^{125} and the antiserum globulin with I^{131}. These differently labelled globulin solutions were mixed so that the mg/cc of protein were equal and the ratio of I^{125} to I^{131} (in counts per min) injected was 253 to 1. *In vivo studies.* Male or female Wistar white rats of approximately 200 g weight were used. Globulin containing 1×10^6 counts per minute was injected intravenously into the tail vein of an unanesthetized rat. For the intrathecal injections the rat was anesthetized in a large jar with ether and the cisternum punctured with a 26 gauge needle attached to a tuberculin syringe. When the globulin was injected subarachnoidally, the volume was limited (from 0.25 to 0.50 cc) so that the count varied from 1×10^4 to 1×10^7 counts per minute. At intervals varying from 1 hour to 96 hours after injection, the rats were decapitated, the blood allowed to flow into a dish containing heparin, and the organs removed and weighed on a Roller-Smith balance and placed in plastic tubes. With brain, kidney and spleen, the whole organ was used for counting; with blood 1 cc and with liver 1 g was used. Counting was done in a Baird-Atomic 810B well using a Baird Atomic 250 Analyzer and 132 Scaler. The counting interval was sufficient to have the values at the 5% level as derived from Loevinger and Berman's table (11).

The counts were calculated as the number per minute per 100 g rat. This represents the radioiodine concentration in terms of per cent of dose in an amount of organ equivalent to 1% of the rat's weight.

Results. Distributions of the normal and antiserum globulin labelled with I^{131} after intravenous administration are shown in Table I. The data were obtained from 2 rats for each time interval chosen and there is no localization of the anti-rat brain globulin in any of the organs tested. In fact, there is greater localization of the normal serum globulin in all the organs.

The amount of iodinated globulin found in brain after the intravenous injections was so small that the subarachnoid route was used

TABLE II. Distribution of I^{131} Labelled Anti-Rat Brain Globulin and Normal Rabbit Globulin in Rat Organs After Intrathecal Injection.

Date	No. of rats	Interval (hr)	% of dose per gram of tissue per 100 g rat			
			Blood	Brain	Liver	
7/11/62	2	1	2.89	1.08	1.03	Normal
	2		2.68	.49	1.14	Anti
			1.07	2.20	.90	Ratio N/A
8/ 2/62	4	2	5.13	2.78	1.34	Normal
	4		5.49	2.70	1.43	Anti
			.93	1.03	.94	Ratio N/A
7/11/62	2	3	2.94	.67	.81	Normal
			1.08	.33	.38	Anti
			2.72	2.03	2.13	Ratio N/A
8/ 2/62	5	4	6.30	2.99	1.17	Normal
	5		4.86	3.38	1.11	Anti
			1.29	.89	1.04	Aatio N/A
8/ 8/62	6	4	2.00	.57	.88	Normal
			2.98	.54	1.32	Anti
			.67	1.05	.67	Ratio N/A
12/ 4/62	4	4*	5.26	4.83	3.13	Normal
			4.54	2.57	2.40	Anti
			1.16	1.88	1.30	Ratio N/A
11/26/62	5	5	.82	.43	.49	Normal
			1.04	.35	.26	Anti
			.78	1.22	1.88	Ratio N/A
9/12/62	10	18	4.43	.48	.95	Normal
			3.31	.40	.83	Anti
			1.33	1.20	1.11	Ratio N/A
12/12/62	4	24	.77	.48	.44†	Normal
			1.18	.35	.61	Anti
			.65	1.36	.72	Ratio N/A
3/22/63	5	72	—	.079	.096	Normal
			—	.035	.097	Anti
			—	2.25	1.0	Ratio N/A
1/14/63	7	96	0	0	0	Normal
			0	0	0	Anti

* Injected 6 days after a previous unlabelled globulin injection.
† Kidney.

for all further experiments. The results of such injections are shown in Table II. A larger number of rats was used and in 9 out of 10 experiments there is greater localization in the brain of the normal serum than of the antiserum. In the liver, in 5 out of 10 cases, the distribution shows greater localization of normal than antiserum and in 4 out of 10 cases the reverse is true. In the remaining case the distribution is equal. Lengthening the time between injection and sacrifice in these experiments did not allow more anti-rat brain globulin to localize in brain.

To eliminate the possibility that individual differences between rats caused the variations, the 2 types of globulin were each labelled with a different isotope and a mixture of the 2 was injected into the same rat. Table III shows that after 18 hours the I^{125} labelled normal globulin is present in the brain in larger amounts than the I^{131} labelled anti-rat brain globulin. The ratio of I^{125} to I^{131} in the brain was 429 to 1, in blood 477 to 1, and in liver 276 to 1 compared with 253 to 1 in the dose injected.

Discussion. The finding that there is less

TABLE III. Distribution of I^{125} Labelled Normal Globulin and I^{131} Labelled Anti-Rat Brain Globulin After Intrathecal Injection into Same Rat.

Date	No. of rats	Interval (hr)	% of dose per gram of tissue per 100 g rat Blood	Brain	Liver	
2/15/63	11	18	1.23 ± .14*	.68 ± .18	.51 ± .12	Normal
			.61 ± .13	.35 ± .11	.46 ± .10	Anti
			2.02	1.94	1.14	Ratio N/A

* Numbers after ± sign are standard deviation. When compared at the .05 level using the student "t", the differences between the means were signficant for brain and blood, but not for liver.

localization of antiserum than of normal serum in brain would seem to correlate with other experimental work in which it was found that it was possible to damage organs such as kidney, pancreas, colon and thyroid (1-5) with their corresponding antisera but that similar damage could not be produced by anti-brain sera(6). It would appear that the brain has a more effective barrier or a more rapid clearing mechanism for antibody.

Summary. (1) Anti-rat brain globulin labelled with I^{131} does not localize in brain after either intravenous or intrathecal injections. (2) Normal rabbit I^{131} globulin seems to localize more in brain than anti-rat brain globulin labelled with I^{131}. (3) When I^{125} labelled normal globulin and I^{131} labelled antiserum globulin were injected into the same rats, the ratio of amount of antiserum globulin in rat brain after 18 hours was half the amount of normal globulin present. (4) time between injection and sacrifice did not change the ratio. (5) The antiserum does not localize in the liver.

We are indebted to Dr. Henry N. Wagner for procedural suggestions and to Mr. Andrew Livingston for technical assistance.

1. Masugi, M., *Beitr. Path. Anat.*, 1934, v92, 429.
2. Baxter, J. H., Goodman, H. C., *J. Exp. Med.*, 1956, v104, 467.
3. Armin, J., Grant, R. T., Wright, P. H., *J. Physiol.*, 1960, v153, 146.
4. LeVeen, H. H., Falk, G., Ostroff, G., Calem, W. S., *Surg. Forum*, 1960, v11, 3.
5. Witebsky, E., Rose, N. R., *J. Immunol.*, 1956, v76, 408.
6. Kolb, L. C., Bolton, B., *J. Neurol. and Psychiat.*, 1940, v3, 111.
7. Williams, C. H., Barnes, F. W., Jr., Gantt, W. Horsley, *6th Res. Conf. on Chemother. in Psychiat.*, 1961, vVI, 171.
8. Williams, C. H., Barnes, F. W., Jr., *Dis. Nerv. Syst.*, in press.
9. Jager, B. V., Nickerson, M., *J. Biol. Chem.*, 1948, v173, 683.
10. Goodland, R. L., Wolfe, D. E., Grimaldi, A., Spar, I. L., Bale, W. F., *UR-476 A.E.C. Report*, 1957.
11. Loevinger, R., Berman, M., *Nucleonics*, 1951, v9, 126.

Received May 1, 1963. P.S.E.B.M., 1963, v113.

Relation Between Blood Level of Corticoids and Their Inhibiting Effect on the Hypophyseal Stress Response. (28442)

P. G. SMELIK† (Introduced by C. H. Sawyer)
Department of Pharmacology, University of Groningen, The Netherlands

The negative feed-back action of adrenocortical hormones on the corticotropic function of the pituitary gland is generally thought to be effected by changes in blood corticoid levels. This implies that an inverse relationship would exist between concentration of corticoids present in the blood and amount of ACTH produced by the adenohypophysis in response to stress. Recently Yates *et al.*(1) have reemphasized this correlation in stating that the increments in plasma corticoid concentration produced by noxious stimuli "are dependent in a predict-

† Present address: University of Utrecht, Holland.