this reason, the fetal brain was examined as a whole. As a result, the sections prepared for spectroscopy included mainly the immature forebrain structures which even in the adult state show the lowest concentration of the lipid fraction.

The increase of the fraction in the fetus following maternal irradiation may be related to the destructive effect of x-rays upon the germinal areas of the forebrain and reflect the results of a chemical breakdown of embryonic cell elements. The formation of peroxides in the skin lipids(7) of x-ray irradiated animals and x-ray irradiated linoleic acid *in vitro*(8), as it might affect the bonding of lipids to protein, may prove a fruitful avenue for further study of the mechanism of the results obtained.

It is expected that these results may contribute to the elucidation of the greater radiosensitivity of the developing brain recently demonstrated by Hicks and confirmed by our own histologic studies. It is also felt that infrared spectroscopy may be valuable for investigation of other complex problems involving lipids in the brain.

Summary. 1. Frozen and dried sections of brain tissue, brain extracts, and pure compounds related to lipid metabolism are studied by infrared spectroscopy. 2. The characteristic infrared absorption bands are tentatively assigned to certain lipid structures. 3. An amide-free lipid fraction is studied in adult and developing brain. 4. Adult and developing brain tissues show different infrared spectra. These differences are in part caused by different content of amide-free lipids. 5. X-ray irradiation decreases the amide-free lipid fraction in the adult and increases that fraction in developing brain.

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Correction of Anemia Following Hypophysectomy by Administration of Cobalt.* (19660)

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Polycythemia can be produced in normal rats by placing the animals in an environment of reduced oxygen tension(1) or by the injection of cobalt(2). Because cobalt is thought to produce polycythemia by interference with oxidative mechanisms(3,4) and because hypophysectomy interferes with the usual increase in red cells and hemoglobin in response to an environment of reduced oxygen tension(5,6) it was thought of interest to see if hypophysectomized rats could respond to cobalt with the production of an increased circulating red cell volume.†

It has been previously shown that 0.05 mg of cobalt and 0.1 mg of iron per 100 g of body weight per day for 60 days is sufficient to produce a 50% increase in the total circulating red cell volume of normal rats(7).

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† An abstract has recently appeared by Crafts(11) in which he reports that administration of cobalt repairs anemia of hypophysectomy.

TABLE I. Hypophysectomized Female Long-Evans Rats Uninjected or Injected Intraperitoneally for 53 Days with .07 mg Cobalt and .1 mg Iron per Day.

	Body wt, g	Hct., %	Hgb. conc., g/100 ml	TRCV, ml/100 g body wt	Total circ. Hgb., g/100 g body wt	% nucleated red cells in marrow
Normal controls	185	52	15.8	2.72	.827	
	199	50.7	15.6	2.16	.663	
	184	49	14.6	2.74	.816	
	187	39.6	12.2	1.89	.583	
Avg	189	47.8	14.6	2.38	.722	
Uninj. hyped	94	34.1	10.8	1.50	.477	31
	90	32	9.8	1.56	.479	37
	107	30.8	9.6	1.25	.392	39
Avg	97	32.3	10.1	1.44	.449	36
Cobalt inj. hyped	80	42.8	12.6	2.31	.679	29
	96	43.7	13.4	2.06	.633	34
	89	47.1	14.4	2.43	.739	33
Avg	88	44.5	13.5	2.27	.684	32

TRCV = total circulating red cell volume; hyped = hypophysectomized.

Methods. Five female rats of the Long-Evans strain, hypophysectomized 5 months previously at 28 days of age, were injected intraperitoneally with 0.07 mg cobalt as cobaltous sulfate and 0.1 mg of iron as ferric chloride per 100 g of body weight daily for 53 days. The ferric chloride was added so that sufficient iron would be available for the formation of hemoglobin. Four normal rats and 5 uninjected, hypophysectomized rats were kept as controls. Two of the injected and 2 of the uninjected animals died during the period of the experiment. At the end of the injection period the total circulating red cell volumes were determined by the Fe^{59} labeled cell dilution method. The labeled cells were obtained from a Long-Evans donor previously injected with Fe^{59}. The experimental animals were injected through the saphenous vein with 0.1 ml of donor blood containing 0.03 μc Fe^{59}. After allowing 6 minutes for the blood to mix, the abdomen was opened and a sample of blood was drawn from the vena cava into a heparinized syringe. A known volume of this blood was pipetted into a vial and its activity counted directly in a scintillation counter(8). The total blood volume was calculated from the fraction of the injected activity recovered in this sample. The hematocrits were determined in Wintrobe tubes. The total blood volume thus calculated multiplied by the hematocrit gave the total circulating red cell volume. The total circulating red cell volumes were then corrected for the body weights of the animals and the results are presented in terms of ml of red blood cells per 100 g body weight. The hemoglobin concentration was determined by the method of Turner(9). Marrow smears were made from the shaft of the femur and the percentage of nucleated red cells was obtained by counting 500 cells from each smear. At autopsy the pituitary site was examined under a binocular dissecting microscope to determine the completeness of hypophysectomy, and the adrenals, thyroid, and testes were weighed as additional evidence of the completeness of hypophysectomy.

Results. The results are summarized in Table I. The uninjected rats showed the anemia typical of hypophysectomy(10). The cobalt injected hypophysectomized rats showed a 58% increase in total red cell volume per 100 g body weight and a 52% increase in grams of circulating hemoglobin per 100 g body weight resulting in a normal total red cell volume and circulating hemoglobin. This 58% increase in total red cell volume is of the same magnitude as the 50% increase seen in normal rats injected with cobalt(7); however, in the latter case this results in the development of a polycythemia while in the former it merely restores the total red cell volume to a normal level. The per-

centage of nucleated red cells in the marrow smears was not increased. This was also true of normal rats injected with cobalt(7). This does not mean, however, that there was not an absolute increase in the number of red cell precursors in the marrow since sections of the marrow were not studied.

Conclusions. The anemia of hypophysectomy was repaired by the administration of cobalt. The increase in red cell volume was of the same magnitude as that reported in normal rats. Apparently the pituitary is necessary for a normal erythropoietic response to hypoxia but is not necessary to obtain a normal erythropoietic response from cobalt. In this way the mechanisms responsible in each case appear to differ.

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A Simple and Sensitive Histochemical Method for Calcium. (19661)

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A histochemical method for staining calcium should be both specific and sensitive. The commonly used Von Kossa silver nitrate stain(1) has great sensitivity but lacks specificity for calcium: its use depends upon the fact that it stains the anions usually in association with calcium, namely phosphate and carbonate. Some of the alizarin dyes were shown by Cameron(2) to have considerable specificity but lacked sensitivity, a defect that has not been remedied by a number of modifications(3-6). Because of low sensitivity this test was found to be inapplicable to the study of early pathological calcification during the period when only minute foci were present.

In conjunction with chemical and anatomical studies(7,8), investigations were undertaken aiming at a stain that was specific, sensitive, and simple. These studies have led to the development of an alizarin staining technic which is almost as sensitive(8) as the Von Kossa and yet which is specific for calcium under the usual conditions of normal and pathological calcification. With it, studies have shown very early intracellular calcification at a time when the Von Kossa test was completely negative, thereby revealing the inadequacy of the latter as an index of the calcium present. Calcium deposits have been demonstrable in tissues from 12-48 hours before microanalytical procedures revealed the presence of excess calcium. Its use with more heavily calcified tissues has shown that the technic gives a satisfactory relative estimate of the amount of calcium present, correlating well with quantitative estimates.

Reagents. 1. Fixative—95% ethyl alcohol. 2. 1% alizarin red S† (sodium alizarin sulfonate) in 0.10% NH_4OH, final pH 6.3-6.5. Stir 0.5 g of the dye into 45 ml of distilled H_2O so that only a few small grains of dye remain undissolved. Add 5 ml of a 1:100 dilution of C.P. 28% NH_4OH slowly with constant stirring. The pH which results is

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† Obtained from National Aniline Division, New York City.