TABLE I.
Changes Induced by Injections of Placental Extracts.

Animal No.	Type	Inj. period, days	Rat wt., gm.	Ovary wt., mg.	Hyp. wt., mg.	% of Cell Types				Sections counted	Cells counted
						Type I	Type III Gran.	Type III Non-gran.	Type IV		
228	Cont.	—	75	27	3.0	37.0	10.0	—	53.0	5	2,828
227	Exp.	6	72	83	3.5	34.2	3.6	4.5	57.7	3	918
231	Cont.	—	83	31	3.5	40.9	10.6	0.2	48.3	5	2,164
232	"	—	89	27	3.0	35.8	7.0	—	57.2	5	1,504
229	Exp.	4	74	91	4.0	32.5	4.1	5.9	57.5	5	1,733
230	"	4	77	87	4.0	31.4	4.1	6.0	58.5	5	1,849
233	"	6	72	100	4.5	32.3	6.9	5.6	55.2	5	2,043
307	Cont.	—	69	23	3.0	32.4	6.5	—	61.1	4	1,177
308	"	—	73	27	3.0	29.8	6.8	—	63.4	4	973
309	Exp.	6	67	83	4.0	27.5	2.5	5.5	64.4	5	777
310	"	6	68	77	4.5	25.0	3.0	6.0	66.0	4	901
311	" (Cast.)	6	69	—	3.5	33.6	10.3	—	56.1	4	1,200
312	" (Cast.)	6	70	—	3.5	33.0	10.0	—	57.0	4	1,015
351	Cont.	—	73	23	2.5	42.2	4.5	0.5	52.8	5	2,174
352	Exp.	6	70	79	3.5	30.6	2.9	6.6	59.9	4	1,530
353	Cont. (Cast.)	—	73	—	3.0	38.9	12.5	—	48.6	4	1,770
354	Exp. (Cast.)	6	70	—	3.0	36.1	11.5	0.7	51.7	4	1,972
‡	Total of sections studied and cells counted									177	65,432

dark blue granules. In the injected animals, these cells were larger but a great majority of them had lost all or a greater part of their granular material (Table I). This was a constant finding in all the injected animals. It was found that castration of the injected animals 2 to 3 days before the beginning of the injection period prevented this loss of granular material from the basophilic elements.

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Presence and Significance of Choline in Cortico-Adrenal Extract.

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The widespread occurrence of choline and its compounds in the normal organism has been regarded as an indication that it may

‡ Editor's Note: Part of table is here published to meet needs of condensation.

have some functional significance. Choline is normally found in the brain, blood, bile, intestine and adrenal cortex; as well as in extracts of the spleen, lymph nodes, thymus, pancreas, etc. In spite of the many studies that have been made, the rôle of choline in health and disease is still a matter of speculation. The antagonistic action between choline and epinephrine is of particular interest because of the normal presence in the adrenal cortex of the former, a powerful parasympathetic stimulant, and the elaboration by the adrenal medulla of the latter, a sympathetic stimulant of the highest order. The humoral possibilities of choline and acetylcholine have been widely advanced, the former as the hormone of intestinal movement by Magnus and LeHeux, and the latter as "Vagusstoff". a possible circulatory hormone, by Loewi and others. It has been reported that choline is converted into acetylcholine by virtue of the ability of the body to supply the acetyl group.[1]

We have analyzed various cortico-adrenal extracts made by the method of Swingle and Pfiffner and have found them all to contain considerable quantities of choline. Four different tests were used on each sample of extract: 2 microscopic tests, a precipitation test, and a color test. We have studied Eschatin (Parke, Davis & Co.) and several different extracts made by Harrop *et al.** In each case, these extracts were found to give strongly positive choline results. Further quantitative work in this connection is in progress.

The question arises, is choline the hormone of the adrenal cortex or is it present in cortico-adrenal extract as a potent, disregarded impurity? To eliminate the former possibility 40 young rats were used in 4 series of experiments, and an attempt was made to maintain life with choline compounds following adrenalectomy. The untreated adrenalectomized controls all died within 3 days. Although some of the choline-treated rats survived somewhat longer than the controls, the results indicated that choline cannot maintain life in adrenalectomized rats and hence must be present in extracts of the adrenal cortex as an impurity which must be removed. Recent investigators attempted unsuccessfully to remove completely the epinephrine from their extracts. No attention has been directed to the presence of choline and its compounds. Acetylcholine has been reported to be hundreds of times more active in causing a fall in blood pressure than is epinephrine in causing a rise in blood pres-

[1] Numerous references may be found in the papers of Dale, H. H., *Lancet*, 1929, **1**, 1285; and Riesser, O., *Arch. f. Exp. Path. u. Pharmakol.*, 1931, **161**, 34.

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sure.[2] This antagonism between the pressor effect of epinephrine and the depressor effect of acetylcholine renders the application of the commonly used method for assaying the epinephrine content of cortico-adrenal extract open to question. Thus Hunt and Taveau[2] have shown that a mixture of 1 cc. of 1:2,500,000 acetylcholine plus 1 cc. of a 1:200,000 solution of epinephrine manifested an active depressor effect instead of the pressor effect anticipated from this high concentration of epinephrine. Hunt also found that acetylcholine, when injected intravenously in the cat in such extraordinarily small doses as 0.000,000,002, 4 mg. per kg., brought about a notable decrease in the blood pressure.[3] The presence of so potent an impurity in cortico-adrenal extract should not be disregarded.

A review of the recent literature indicates that many of the properties and results attributed to the hormone of the adrenal cortex simulate typical choline action and may very well be the result of injection of choline-containing extracts. Choline, a profound stimulant of the parasympathetic nervous system, has been reported to influence markedly carbohydrate metabolism, cause glycogen mobilization, alter the blood picture particularly with respect to the quantitative blood cellular arrangement, augment water excretion, increase respiratory metabolism, and bring about many other results—all of which have been recently attributed to the hormone of the adrenal cortex by various investigators.

We have also analyzed extracts made by the method of Grollman and Firor* and have been unable to detect choline. No epinephrine could be found in these extracts by chemical or biological methods, indicating that epinephrine is present only in traces, if at all. The relative absence of noxious by-products in the extracts made by this latter method as compared to those made by other current methods has also been emphasized by these authors.[4]

[2] Hunt, R., and Taveau, R. de M., Bull. 73, Hyg. Lab., U. S. Public Health and Mar. Hosp. Service, Wash., 1911.

[3] Hunt, R., *Am. J. Physiol.*, 1907, **45**, 197.

[4] Grollman, A., and Firor, W. M., *J. Biol. Chem.*, 1933, **100**, 429.