size and other abnormalities indicative of interference with normal development.

The results of a more complete study under way(8), including histological, histochemical and reversal attempts, support the view that these effects reflect the interference with purine and pyrimidine utilization by the embryo rather than being due to generalized toxic reaction.

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A Technic for Determination of Adrenocorticotrophin in Blood.* (19403)

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The oxycellulose technic of Astwood *et al.* (1) for the isolation of adrenocorticotrophin (ACTH) from pituitary has been successfully adapted to the quantitative analysis of ACTH in rat blood.

Methods. Collection of blood. Male rats of the Sprague-Dawley strain, weighing 150 to 300 g, were employed. Each rat was anesthetized with ether and bled from the abdominal aorta into a heparinized syringe. Four volumes of glacial acetic acid were immediately added to the sample and the mixture was thoroughly stirred. Pools of blood-glacial acetic acid mixtures were processed for ACTH as described below.

Technic for concentration of blood ACTH. 1) The mixture of blood and glacial acetic acid is gently stirred at 70-75°C for 30 minutes in a water bath and then transferred to a large pyrex cylinder. 2) To each ml of the above mixture are added 13.0 ml distilled water with constant stirring. 3) 0.1 g Hyflo-Super-cel per ml of blood is stirred into the mixture which is then allowed to stand for 24 hours. The Super-cel settles to the bottom of the cylinder. 4) The supernatant is siphoned into a pyrex cylinder for further processing; the Super-cel is discarded. 5) A slurry of prepared† oxycellulose‡ (0.04 g per ml blood) is added to the supernatant with continuous stirring; stirring is continued for 24 hours to allow for complete "adsorption" of ACTH on oxycellulose. 6) The oxycellulose is now allowed to settle for 24 hours. 7) The supernatant is siphoned off and discarded; the oxycellulose is collected on a course, sintered-glass funnel and washed with 30-ml quantities of 0.1 N acetic acid until the washings are

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† A weighed amount of oxycellulose is washed into a sintered-glass funnel with three 25-ml quantities of 0.1 N acetic acid, then washed successively with 25 ml distilled water, 25 ml 0.1 N HCl, 25 ml distilled water and, finally, with three 25-ml quantities 0.1 N acetic acid. A slurry of the washed oxycellulose is prepared with 15 to 30 ml 0.1 N acetic acid.

‡ Samples of oxidized cellulose (11-12% COOH) were generously supplied by the Research Laboratories, Tennessee Eastman Co.

colorless. 8) ACTH is now eluted from the oxycellulose with 0.1 N HCl. The oxycellulose is washed into a beaker with an appropriate volume of 0.1 N HCl. The slurry is stirred frequently for one hour and then filtered by gravity or by gentle suction on the sintered-glass funnel. Two additional elutions are carried out and the eluates are combined. The minimum volume of 0.1 N HCl to make a slurry of workable consistency in the first elution is 15 ml per 4 g of oxycellulose. The volume of 0.1 N HCl used in the second and third elutions is determined by the dose to be injected into the assay rat. For example, if 100 ml blood are processed and the oxycellulose is eluted initially with 15 ml and then eluted twice with 5-ml quantities of 0.1 N HCl, each ml of combined eluate is equivalent to approximately 4 ml blood.

Method of assay. ACTH was assayed in hypophysectomized male rats by the adrenal ascorbic acid-depletion method previously reported from this laboratory (2). Unknown samples were compared with freshly prepared solutions of U.S.P. Standard ACTH in every assay. Results are expressed in U.S.P. milliunits (mu) of ACTH. One mu is one thousandth of a U.S.P. Unit of ACTH which is defined as the activity of one mg of the International Standard ACTH (La-1-A).

Results. Hypophysectomized rat blood. Three separate pools of blood obtained from rats hypophysectomized 24 hours prior to bleeding were assayed for ACTH. No significant ascorbic acid depletion occurred when a dose§ of eluate equivalent to 18 ml hypophysectomized rat blood was administered to the test animal. (Table I, Assays 1 and 2.)

Recovery of exogenous ACTH from hypophysectomized rat blood. A pool of blood was collected from rats 24 hours after hypophysectomy. Fifty U.S.P. mu of Standard ACTH were added to the blood immediately prior to processing. The eluate was diluted with 0.01 N HCl so that the administered dose contained 1.0, 0.5, or 0.25 mu of added ACTH. The results (Table I, Assay 1) indicate that 82 $\pm$ 12% of the added hormone was recovered.

Recovery of endogenous ACTH from adrenalectomized rat blood. The work of Gemzell *et al.* (3) demonstrated that plasma from adrenalectomized rats has a high concentration of ACTH. Preliminary studies employing the oxycellulose technic confirmed this observation and indicated that it would be possible to analyze the blood of adrenalectomized rats for ACTH without concentration. A comparison has been made of the estimate of blood ACTH of adrenalectomized rats by direct transfusion with that obtained by the oxycellulose technic.

Oxycellulose technic. Male rats weighing 150 to 300 g were bilaterally adrenalectomized and given a solution of 0.9% sodium chloride to drink. One week after adrenalectomy blood was collected and processed as described above. The equivalents of 12, 6, 3 and 0.75 ml of blood were administered to the test animals. The results of 2 assays (No. 2 and No. 3) indicate that each 100 ml of blood from these adrenalectomized rats contains 12.0 $\pm$ 2.5 mu and 10.3 $\pm$ 2.0 mu of ACTH, respectively.

Transfusion. Male rats, bilaterally adrenalectomized and maintained as described above, served as donors. In these experiments blood was not pooled; each test animal received blood from a single adrenalectomized donor. Immediately after the sample was obtained, a dose of 1.5, 3, 5 or 6 ml was injected via the saphenous vein of the hypophysectomized test animal. The results (Table I, Assays No. 3, 4 and No. 5) indicate that each 100 ml of blood from adrenalectomized rats contains about 15 mu of ACTH. Comparison of the value obtained by direct transfusion with that obtained by the oxycellulose technic suggests that the method recovers at least 70% of blood ACTH. The fact that transfusion of a dose of 6 ml of hypophysectomized rat blood into the test animal (Assay 5) induced no depletion of adrenal ascorbic acid eliminates the possibility that a large volume of blood *per se* produces a false positive response in the method of assay for ACTH.

Recovery of endogenous ACTH from intact rat blood. Two separate pools of blood were

§ Doses represent quantity administered per 100 g body weight of assay rat.

TABLE I. Assay Blood ACTH.

Assay No.	Sample	Dose/100 g assay rat	Response, mg ascorbic acid/100 g adrenal: Individual	Avg	Estimate of ACTH recovery or potency with S.E.
1	U.S.P. Std ACTH	1 mu	94, 132, 178, 114, 164, 139, 102, 138	133	
		.5	72, 131, 56, 129, 117, 119, 146	110	
		.25	36, 21, 52, 34, 77, 83, 84, 35	53	
	Hypox blood #1 (4 g oxycel./100 ml blood)	18 ml	−16, −15, −9	−13	0 mu/100 ml*
	Hypox blood #2 (4 g oxycel./100 ml blood)	18	26, −2, 0	8	0 mu/100 ml*
	Hypox blood + ACTH (4 g oxycel./100 ml blood)	1 mu	133, 131, 136, 112, 86, 114, 134, 147	129	82 ± 12% recovery
		.5	48, 97, 63, 112, 64, 93	86	82 ± 12% recovery
		.25	70, 99, 20, 38, 21, 58, 28, 67	50	82 ± 12% recovery
2	U.S.P. Std ACTH	1	148, 204, 128, 194, 148, 207	172	
		.5	47, 135, 106, 170, 129, 93	116	
		.25	6, 27, 20, 45, 88	37	
	Adrenex blood (4 g oxycel./100 ml blood)	12 ml	177, 148, 166, 186	169	12±2.5 mu/100 ml
		3	54, 97, 72, 67, 129	84	12±2.5 mu/100 ml
		.75	23, 12, 12, 57, −36, 20, 47	19	12±2.5 mu/100 ml
	Hypox blood (4 g oxycel./100 ml blood)	18	−8, 5, 22, 26	11	0 mu/100 ml*
3	U.S.P. Std ACTH	1 mu	199, 212, 127, 255, 160, 171, 173, 157	182	
		.5	163, 195, 99, 187, 111, 169, 117, 144	148	
		.25	63, 79, 49, 26, 84, 99, 114, 121	79	
	Adrenex blood (4 g oxycel./100 ml blood)	6 ml	216, 138, 190, 150, 203, 195, 119	173	10.3±2 mu/100 ml
		3	126, 166, 135, 127, 109, 147, 55	123	10.3±2 mu/100 ml
	Adrenex blood (transfused)	5	159	159	8 to 17 mu/100 ml
		3	161, 69, 75, 90, 55, 138, 98, 102, 179, 149	112	8 to 17 mu/100 ml
4	U.S.P. Std ACTH	1 mu	143, 156, 138, 133, 124, 127, 140, 155	140	
		.5	143, 100, 86, 107, 121, 95	109	
		.25	63, 36, 47, 32, 31, 35	41	
	Adrenex blood (transfused)	6 ml	99, 74, 123, 109, 144, 109, 182, 105, 198, 173	132	16.1±2.1 mu/100 ml
		3	96, 74, 129, 108, 95, 115, 103	103	16.1±2.1 mu/100 ml
5	U.S.P. Std ACTH	1 mu	204, 104, 175, 79, 138, 185, 151	148	
		.5	105, 138, 161, 126, 86, 145	127	
		.25	90, 138, 135, 55, 77, 37, 77	87	
	Hypox blood (transfused)	6 ml	−41, 1, −34, 17, 1	−11	0 mu/100 ml*
	Adrenex " "	6	127, 165, 105, 163, 170, 103, 127	137	14±4.3 mu/100 ml
		3	113, 82, 114, 138, 101	110	14±4.3 mu/100 ml
		1.5	50, 110, 91, 140	98	14±4.3 mu/100 ml
6	U.S.P. Std ACTH	1 mu	68, 163, 159, 98, 176	133	
		.25	36, 87, 46, 47, 62, 56	56	
	Intact blood #1 (4 g oxycel./100 ml blood)	12 ml	60, 60, 67, 56, 58	60	2.1 ± .6 mu/100 ml
		6	19, 17, 1, 16, −12	7	2.1 ± .6 mu/100 ml
	Intact blood #2 (4 g oxycel./100 ml blood)	12	103, 82, 86, 52	81	2.9 ± .8 mu/100 ml
		6	15, 43, 47, 3, 15	25	2.9 ± .8 mu/100 ml
7	U.S.P. Std ACTH	1 mu	195, 118, 145, 112, 140, 110	137	
		.5	71, 123, 60, 82, 149	97	
		.25	25, 19, 35, 25, 33	27	
	Adrenex blood (2 g oxycel./100 ml blood)	6 ml	72, 124, 56, 140	98	11.1 ± 2.2 mu/100 ml
		3	105, 59, 70, 73, 21	66	11.1 ± 2.2 mu/100 ml
	Same but .2 g	6	24, 17, 54, 11, 4, 19	22	< 25% recovery*†
		3	−31, −4, −24, −37	−24	< 25% recovery*†
	Same but .02 g	6	25, 12, −1, −50, 0, 39	4	< 25% recovery*†
		3	−7, −23, −20, −23, 5, −16	−14	< 25% recovery*†

* Estimate made without analysis of error. † Assuming 2 g oxycellulose recovers 100%.

collected and processed. The eluate of each pool was administered in doses equivalent to 6 and 12 ml of blood. The results are recorded in Table I, Assay 6. The responses obtained for the two pools are in excellent agreement. Statistical analysis indicates that intact rat blood obtained by acute exsanguination contains 2 to 3 mu ACTH per 100 ml.

Recovery of endogenous ACTH from adrenalectomized rat blood with various quantities of oxycellulose. In the experiments reported above, 4.0 g oxycellulose were added to each 100 ml blood. The results of Assay 7 indicate that 2.0 g oxycellulose per 100 ml blood are as effective as 4.0 g in recovering blood ACTH (*cf.* potency adrenalectomized rat blood as determined by Assays 2 and 3 with potency as determined by Assay 7). However, quantities of 0.2 and 0.02 g are obviously insufficient.

Discussion. The adrenal ascorbic acid-depletion technic is not sensitive enough unequivocally to detect ACTH in unconcentrated blood of normal man or rat. Taylor *et al.*(4) did not find any significant depletion of adrenal ascorbic acid of the hypophysectomized rat after the administration of 2 to 3 ml of serum from normal man and from patients with Cushing's syndrome. In this laboratory (Richards)(5), 4 to 6 ml of blood obtained from the abdominal aorta of the intact rat failed to induce a significant depletion of adrenal ascorbic acid. Taylor *et al.*(4) have presented data which suggest that 2 to 3 ml of serum from individuals with adrenocortical insufficiency contain a detectable quantity of ACTH. It is apparent that biological activity must be concentrated in order to determine with accuracy the quantity of ACTH in blood. Cooke *et al.*(6) and Bornstein and Trewhella(7) have described technics for concentration of biological activity in plasma. However, they did not present experimental data to indicate the specificity or the quantitative recovery to be expected from these methods. Gemzell *et al.*(3) have claimed, without presentation of data, that an acid-acetone technic will quantitatively recover ACTH from rat plasma. In this laboratory, the acid-acetone technic has not been reproducible and has not yielded quantitative recovery of blood ACTH. Moreover, toxicity of acid-acetone concentrates of plasma and, especially, of blood is a decided disadvantage of this method.

The oxycellulose technic as described above satisfies the following criteria for a sound method for the determination of ACTH in blood. It appears to be *specific for ACTH,* as demonstrated by the fact that the blood of hypophysectomized animals induces no depletion of adrenal ascorbic acid in the assay rat. Experimental studies presented above indicate that the method *almost completely recovers ACTH added to blood.* The concentration of ACTH in the blood of the adrenalectomized rat, as determined by the oxycellulose technic, was nearly the same as that determined by transfusion of adrenalectomized rat blood directly into the test animal. These results suggest that the oxycellulose method *almost completely recovers endogenous ACTH.* The eluates have caused *no toxic reactions* in any instance. Limited experience to date suggests that the method is *highly reproducible* and is *applicable to other species* (dog and man¶).

No experimental data are available to justify all the steps enumerated under the description of technic. The technic as described is essentially that employed by Astwood *et al.*(1) for the isolation of ACTH from pituitary. Future developmental studies may make it possible to simplify the procedure for the analysis of blood ACTH. Without reliable information regarding stability of ACTH in blood it was deemed advisable to mix the sample with glacial acetic acid immediately after collection. As a routine procedure, the glacial acetic acid-blood mixture is diluted with water within four hours after collection of the sample.

Blood rather than plasma has been employed for two reasons. First, no information is available on the partition of ACTH between cellular elements and plasma. Second, the time required to separate cells and plasma

¶ Preliminary studies indicate that the blood of patients with Addison's disease has a higher than normal concentration of ACTH.

may be sufficient for inactivation of blood ACTH.

The experimental results reported here are not strictly comparable with those reported by Gemzell *et al.*(3) since the latter analyzed blood from the inferior vena cava. Blood from the abdominal aorta was employed in the present studies. We have confirmed Gemzell *et al.*(3) that blood of the adrenalectomized rat has a greater concentration of ACTH than normal. However, these investigators were unable to detect activity in 448 ml of plasma from intact rats, whereas our data indicate that 12 ml of blood from these animals induce a definite depletion of adrenal ascorbic acid.

Summary. 1. The oxycellulose technic for isolation of ACTH from pituitary has been successfully employed to concentrate and quantitatively to measure blood ACTH. The method a) appears to be specific for ACTH since hypophysectomized rat blood induces no adrenal ascorbic acid depletion in the assay animal, b) almost completely recovers ACTH added to hypophysectomized rat blood, c) almost completely recovers endogenous ACTH, d) yields concentrates which are not toxic to the assay animal and e) is reproducible. 2. It is estimated that rat blood, obtained by acute exsanguination of intact animals, contains 2 to 3 mu ACTH per 100 ml, whereas blood obtained under similar conditions from rats bilaterally adrenalectomized for one week contains 10 to 12 mu ACTH per 100 ml.

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A New Operative Preparation for Production of Peptic Ulcer in the Dog.* (19404)

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Many data have been obtained by previous investigators concerning experimentally induced acid-peptic ulceration of the stomach and juxtapositioned parts of the alimentary canal. However, the methods employed to produce the ulceration are open to serious question. The procedure of duodenal drainage (Mann and Williamson)(1), causes severe nutritional difficulties in the dog leading to inanition. In addition, it has been shown (Storer, Oberhelman, Woodward, and Dragstedt)(2), that gastric secretion is greatly increased by this procedure. We feel that these objections have been satisfactorily overcome by our preparations to be described below. In the human and in the dog most of the secretion of the parietal cells occurs in response to neurogenic (vagal) or hormonal stimulation, the latter arising mainly from the antral mucosa and to a lesser extent from the small intestine. Accordingly, it is not physiologic to evaluate experimentally vagotomy or antrum resection in the dog when an exogenous stimulus to the parietal cells, as histamine, is employed.

We have devised an alternate method of producing experimental peptic ulceration in

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