the dermal rather than the intravenous route. Both these mechanisms may be operative in the development of the late lesions.

Summary. Late lesions with the qualities of a delayed hypersensitivity reaction occur in the skin of rats injected intradermally with rabbit anti-rat tissue sera. These lesions are related to the fixation and persistence of antibodies directed against antigens in the epithelial and mesenchymal basement membranes of skin.

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Effect of Brain Removal in Dogs Previously Subjected to Pituitary Stalk Section.* (29233)

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The output of 17-hydroxycorticoids is elevated in dogs after removal of the brain(1-3). The elevation is due to increased ACTH secretion from the isolated pituitary(2,3), but the cause of this increase is unknown. Brain removal entails disruption of the hypophyseal portal vessels, the principal pathway by which blood reaches the anterior pituitary, and the resultant ischemia might cause a prolonged elevation in rate of ACTH secretion. The posterior lobe remnants left in the pituitary fossa might also play a role because the corticotropin-releasing factor known to be present in this tissue(4) might reach the anterior lobe via the vascular anastomoses between the 2 lobes(5). To explore these possibilities further, we have studied 17-hydroxycorticoid output in the adrenal vein before and after brain removal in dogs in which the pituitary stalk had been sectioned and a plastic plate inserted between the hypothalamus and the pituitary 2 to 10 weeks previously. An abstract reporting the results of some of these experiments has been published(6).

Methods. The pituitary stalk was sectioned via the transtemporal approach(7) in male mongrel dogs anesthetized with pentobarbital. After temporal craniectomy and opening of the dura, the temporal lobe was elevated, the oculomotor nerve was cut, and the hypophyseal stalk was visualized and sectioned with a blunt instrument. A 2-component epoxy polyamide resin (resiweld 620, H. B. Fuller Co.) was mixed and loaded into a small disposable plastic syringe attached to a blunt No. 17 gauge needle. As the temporal lobe and the hypothalamus were gently elevated with a flat-blade retractor, the needle was placed between the hypothalamus and hypophysis, the plunger firmly pressed, and the point of the needle gradually moved about. The freshly mixed resin was viscous, but flowed sufficiently well before hardening to form a solid cap molded over the superior surface of the hypophysis. We have found this method of interposing a barrier between the hypothalamus and the pituitary to be very satisfactory.

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Two of the dogs received 12.5 I.U. per day of ACTH in the post-operative period, stopping the day before cannulation, but the remaining animals received no special treatment. The 17-hydroxycorticoid outputs of the 2 treated dogs did not differ from those of the untreated dogs, so the responses of all were averaged. Two to 10 weeks after stalk section, each dog was anesthetized with pentobarbital and a cannula placed in the right adrenal vein(8). Two adrenal venous blood samples were then collected, one as soon as the cannula was in place and another starting 4 minutes after intravenous injection of 10 mU of ACTH. The response to 1000 mU of ACTH was also determined, either at this time or at the end of the experiment. In one group of dogs, all brain tissue above the midbrain was then removed, leaving the pituitary under the plastic plate. Adrenal venous blood samples were collected at hourly intervals for 2 to 4 hours after brain removal, and the animals were then subjected to a second laparotomy. Adrenal venous blood was collected commencing 5 minutes after the laparotomy. In a second group of dogs, the flank incisions were sutured and the animals returned to their cages. Twenty-four hours later, an adrenal venous blood specimen was collected from each of these unanesthetized dogs. They were then anesthetized with pentobarbital and subjected to brain removal, using the same protocol that was used in the first group of dogs.

All adrenal venous blood specimens were promptly centrifuged and the plasma frozen for subsequent determination of 17-hydroxycorticoid content by the method of Silber and Porter(9). Output of 17-hydroxycorticoids was calculated by multiplying the adrenal venous plasma flow per minute by the 17-hydroxycorticoid concentration. Blood pressure was monitored continuously during and after brain removal in each dog by means of a Statham strain gauge and Grass Model 5 polygraph. Animals which appeared to lose appreciable amounts of blood during the operation were given transfusions of blood from hypophysectomized dogs. Rectal temperature was monitored with a thermistor probe and maintained at or near the normal level by applying external heat when necessary.

TABLE I. Adrenal Venous 17-Hydroxycorticoid Output in Stalk-Sectioned Dogs. Values in parentheses are number of animals.

	17-Hydroxycorticoid output (μg/min $\pm$ standard error)	
	Stalk-sectioned dogs	4 normal dogs
Surgical stress	4.1 $\pm$ .8*(11)	9.8 $\pm$ .6
10 mU ACTH	6.1 $\pm$ .5*(11)	11.1 $\pm$ .7
1000 mU ACTH	7.4 $\pm$ .9 (9)	9.3 $\pm$ .9
24 hr after stress	1.1 $\pm$ .6 (5)	
Restress	5.3 $\pm$1.0 (5)	
After brain removal		
1 hr	2.2 $\pm$ .6 (8)	
2 hr	3.5 $\pm$ .7 (7)	
4 hr	3.7 (2)	
Restress	3.5 $\pm$ .5 (5)	

* Significantly different from corresponding value for normal dogs ($p < .01$).

The hypothalamic tissue removed at operation and the pituitary tissue under the plastic plate were serially sectioned, stained and examined microscopically. The right adrenal glands were removed, cleaned and weighed. In 3 instances, the adrenals were sectioned and examined histologically. Four normal dogs subjected to adrenal venous cannulation served as controls for these experiments.

Results. A total of 12 dogs in which the pituitary stalk had been sectioned 15 to 70 days previously were studied. The adrenals of one of these dogs were unresponsive to ACTH, so it was excluded from the series. The remaining 11 dogs had 17-hydroxycorticoid outputs after 10 mU of ACTH of 3.2 to 8.7 μg/min, and following 1000 mU ACTH, outputs of 4.0 to 13.1 μg/min. Mean outputs are shown in Table I. Responses of all the animals to stress and 10 mU of ACTH were determined, but the number of animals at each particular point thereafter is less than 11 because 5 of the dogs were studied as chronic cannula preparations, and several of the dogs died during brain removal or shortly thereafter. Blood pressure was generally above 80/40 mm Hg in all the remaining dogs, and in most of them it was in the normal range. The mean weight of the right adrenals of the stalk-sectioned animals (excluding the 2 animals which received ACTH) was 47.0 $\pm$ 4.5 (standard error)

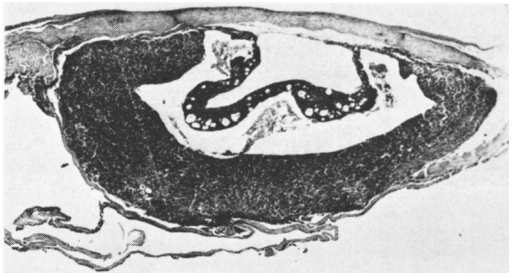

FIG. 1. Sagittal section of tissue under plate between hypothalamus and pituitary in a stalk-sectioned dog. Hematoxylin and eosin, × 8.

mg/kg body weight. The mean right adrenal weight in 10 normal dogs was 54.7 ± 4.8 mg/kg This value is not significantly different from that of the stalk-sectioned dogs ($p > 0.2$). However, in the adrenals of 3 dogs chosen at random for microscopic examination, there was histological evidence of moderate adrenocortical atrophy.

There was a remnant of the pars tuberalis above the epoxy plate in one dog and a few tags of tuberal tissue in another, but in the remaining dogs, no pituitary tissue above the plate was seen in the serial sections of the hypothalamus. There was variable damage to the median eminence in all dogs, but in none was it completely destroyed. The responses in the dogs with remnants of pars tuberalis were no different from those in the other dogs, and there was no apparent correlation between degree of median eminence damage and magnitude of the adrenocortical response. In all animals, there was a dense dural scar above the pituitary, and all neurohypophyseal tissue under the scar was atrophic. Intermediate lobe tissue appeared to be slightly hypertrophied in most instances. There were infarcts in the anterior pituitary in all cases. The infarcts varied in size but in all animals there was some histologically normal adenohypophyseal tissue. In several dogs, there was very little damage to the anterior lobe (Fig. 1). In general, the magnitude of the adrenal response to stress and to ACTH was greater in the dogs with more normal-appearing adenohypophyseal tissue. No portal vessels were seen traversing the scar between the hypothalamus and the anterior pituitary; however, a few small vessels might have escaped detection because India ink was not injected to make the portal vessels stand out.

Discussion. There was some loss of adrenal responsiveness to ACTH in stalk-sectioned dogs, since the output of 17-hydroxycorticoids following administration of 10 mU of ACTH was significantly lower than in the controls, and the response to the small dose of ACTH was substantially less than the response to the large dose. Nevertheless, 17-hydroxycorticoid output following the stress of adrenal vein cannulation in these dogs was appreciable, averaging 67% of the response to 10 mU ACTH. It has previously been reported that 17-hydroxycorticoid output is elevated in stressed dogs during the first 24 hours after stalk section(2,10). The responses in other species vary somewhat, but in general, responses to many stresses are present(11).

Most investigators report that the adrenals of stalk-sectioned animals are atrophic although not as atrophic as those of animals hypophysectomized for comparable periods. In our stalk-sectioned dogs, the adrenals were not markedly atrophic. However, there was some correlation between adrenal size and length of time between stalk section and brain removal, suggesting that more marked adrenal atrophy might have been seen if the animals had been kept alive longer.

Our experience with use of the epoxy-polyamide resin to separate the hypothalamus and the pituitary has been entirely satisfactory. It forms a tough, solid, slightly irregular plate which follows the contours of the pituitary tissue. The method has been especially successful because it permits injection of material anteriorly between the carotid arteries, an area which is sometimes left uncovered when other types of plates are inserted.

The present results seem to rule out liberation of CRF (corticotropin-releasing factor) from posterior lobe tissue or trauma to the adenohypophysis(12) as explanations for the increased 17-hydroxycorticoid output in dogs following removal of the brain. All posterior lobe tissue below the level of the stalk section was atrophic, and the anterior lobe was protected during brain removal by the plastic cap overlying it. The 17-hydroxycorticoid

output values fell to basal levels 24 hours after cannulation, and rose again when the animals were restressed, suggesting that a pituitary-stimulating humoral factor was probably liberated into the general circulation during stress.

It has been argued in the past that CRF from the hypothalamus enters the general circulation in stressed, stalk-sectioned animals (12). However, the 17-hydroxycorticoid output in our dogs remained elevated after the hypothalamus was removed. In view of our work(2), and that of Egdahl(1), it seems unlikely that the central nervous system, the kidneys, or the other abdominal viscera are the source of this pituitary-stimulating factor, but it could be coming from damaged tissues (*e.g.* cranial or abdominal musculature).

Fortier, Harris and MacDonald(13) have suggested on the basis of their stalk-section experiments in rabbits that there are 2 types of stress: "neural" stresses which act through the hypothalamus to increase ACTH secretion, and "systemic" stresses such as surgical trauma which stimulate the anterior pituitary directly. The present results would seem to support this hypothesis. However, it should be emphasized that the response to cannulation of the adrenal vein alone in dogs is blocked by median eminence lesions(14). The present dogs had undergone craniectomy and brain removal as well. Thus, if there is a circulating pituitary-stimulating factor liberated in surgically stressed dogs, it must require extensive surgical trauma, such as that carried out in the present dogs, to cause its liberation. It also appears that such a factor is not secreted in the rat(15).

One note of caution must be injected into the interpretation of the present results. Since the hypothalamus was surgically removed, it was not possible to perfuse the dogs with India ink at the time they were sacrificed or to serially section the hypothalamus and pituitary as a unit. Therefore, regeneration of portal vessels around the plate cannot be absolutely ruled out, even though no vessels were visible in the scar tissue that covered the pituitary. If such regeneration had occurred, the stress responses could have been due to CRF liberated into these vessels, and interruption of them during brain removal could have led to anterior pituitary ischemia. It will be necessary to perform brain removal experiments in dogs with autotransplanted pituitaries to eliminate this possibility completely. At present, however, the best working hypothesis is that in severely stressed dogs, a humoral factor liberated from some part of the body other than the brain (presumably traumatized tissue) is responsible in part for the increase in ACTH secretion.

Summary. The effects on adrenal cortical function of removing the hypothalamus and other neural tissue above the midbrain were determined in 11 dogs in which the pituitary stalk had been sectioned previously and a plastic plate placed over the pituitary. There was a clear-cut response to stress 2 to 10 weeks after stalk section, and 17-hydroxycorticoid output in the unstressed state 24 hours after adrenal vein cannulation (before brain removal) was low. However, following subsequent brain removal, 17-hydroxycorticoid output was elevated. All neurohypophyseal tissue below the plastic plate was atrophic in these dogs. There was no marked adrenocortical atrophy. The results suggest that the stress response in severely stressed dogs is not due entirely to CRF from the hypothalamus, and may be due in part to an ACTH-stimulating humoral agent liberated from traumatized tissues.

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Separation of SV40 from Poliovirus by Extraction with l-Butanol. (29234)

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During experimental work on the concentration and purification of poliovirus using the method described by Schwerdt and Schaffer(1), it was observed that the levels of adventitious SV40 were markedly reduced. A detailed study of the level of SV40 at each step in the process revealed that a sharp reduction in infectious SV40 took place when the first aqueous concentrate was washed with 1-butanol.

In early work on the purification of poliovirus of CNS origin, Bachrach and Schwerdt (2) applied Morton's procedure(3) of butanol extraction for removal of non-viral protein. At pH values between 4.5 and 9.0 and ionic strength of 1.0, the virus remained unaltered in the aqueous layer.

The purpose of the present study is to determine whether the observed reduction of infectious SV40 in the presence of poliovirus was due to selective inactivation or to selective removal from the aqueous phase by the 1-butanol.

Three sets of experiments were done. The first was an examination of the effects of butanol on culture fluids high in SV40 but free from poliovirus. The second was to confirm that neither a virulent nor an attenuated strain of poliovirus was inactivated by treatment with butanol. In the third set, pools of attenuated virus vaccine (Sabin) of all 3 poliovirus types, containing adventitious SV40, were extracted with the solvent.

Also, preliminary observations were made on the effect of butanol extraction on a number of other viruses, particularly those commonly associated with tissue cultures of simian origin.

Materials and methods. Pools of SV40 of high titer were prepared by infecting trypsin-dispersed Cercopithecus monkey kidney cells in suspension, planting the infected cells and incubating the cultures for 8 days. After 3 cycles of freezing and thawing the fluids were harvested. The strain of SV40 used was isolated in these laboratories from a culture of Cercopithecus monkey kidney. It was identified by cross-neutralization with immune rabbit serum prepared against Dr. Hilleman's Strain 45-54(4). When grown in monolayers of Cercopithecus monkey kidney cultures it produces the cytopathic effect typical of Hilleman's vacuolating agent.

The poliovirus cultures, free from SV40, were from routine lots prepared for either Salk or Sabin vaccine. Mixed virus pools of poliovirus and SV40 were obtained from lots of Sabin vaccine rejected because of their contamination with SV40. The SV40 found in these pools was identified serologically.

The various strains of simian virus were isolated from routine tissue culture samples taken during the preparation of both Salk and Sabin vaccines. Three of these strains were submitted to Dr. Hull (Eli Lilly & Co.) for identification. The others are serologically different and are designated by our own simian virus numbers. Pools of high titer have been prepared in monolayer cultures.

Titrations of SV40 were carried out by inoculating fully grown roller-tube cultures of trypsin-dispersed kidney cells from Cerco-