process as stated above, the mechanism of the action might be quite different from that of proflavine in a sense that the action is reversible.

Summary. In the presence of a non-toxic concentration of N^1-isobutylbiguanide hydrochloride, HA production in L cells infected with Sendai virus was reduced to 1/16 of the control. The effect of the compound was reversible, and the presence of the compound through the entire course of infection was necessary and essential for the inhibition. The number of infectious centers was not reduced even when the cell was treated with the compound all through infection, indicating that the compound did not affect the processes of adsorption or penetration of the virus. However, the infected cells remained in the first stage of antigen development in the presence of the compound, *i.e.*, the accumulation of antigens was limited to the juxtanuclear region of cytoplasm and not extended to the entire cytoplasm. It is concluded that the effect of the compound is on the inhibition of the late maturation mechanism of Sendai virus synthesis.

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An Ovarian Ascorbic Acid Depletion Factor in Starch Gel Preparations Following Electrophoresis.* (30469)

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In studying ovarian ascorbic acid depletion (OAAD) activity in the plasma of hypophysectomized cockerels(1), an attempt was made to use starch gel electrophoresis to separate and purify plasma LH. This approach led to an investigation of the OAAD activity in the starch gel preparation itself.

Materials and methods. Starch gels were prepared as reported by Ferguson and Wallace(2) with certain modifications. Partially hydrolyzed potato starch (Connaught Medical Research Laboratories, Toronto) was suspended in a buffer composed of 90% 0.0033 M citric acid, 0.016 M Tris, and 10% 0.02 M lithium hydroxide, 0.076 M boric acid, with a pH at 20°C of 8.1. After heating properly, the gel was poured into a mold on a glass plate formed by plexiglass strips, which were held in place by clips for a final mold size of 12 × 21.7 × 0.6 cm. A mold with 8 slot-formers equidistantly spaced, was placed on the hot gel in such a way as to form 8 slots, 1.2 cm wide, 0.5 cm deep, and 0.1 cm thick, 5 cm from one end of the gel. The gel was cooled at 5°C for at least 30 minutes, the mold carefully removed, hot white petroleum jelly added to the area about each slot, and then 3 drops of the sample material injected into each slot. The entire surface of the gel was then covered with hot petroleum jelly, after which a 2-cm strip of the cooled jelly was removed from each end to allow contact with the tray buffer wicks. Departing from Ferguson and Wallace(2), the tray buffer was 0.0286 M lithium hydroxide and 0.19 M boric acid, which was placed in the cathodal trays and in the anodal tray directly connected to the

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TABLE I. OAAD Activity in Starch Gel Supernatant Suspensions.

Assay No.	Lambda	No. assay animals	Material inj into slot	Treatment of gel supernatant	Mean % depletion (± S.E.)	p‡
8	.19	6	Blank	Heated or untreated	21.6 ± 8.1	<.05
		12	Cockerel plasma	Heated	14.6 ± 4.2*	<.05
		12	" "	Untreated	25.8 ± 4.2*	<.01
11	.54†	10	Albumin or blank	None	20.1 ± 6.5	<.05
		5	No electrophoresis of blank	"	6.0 ± 5.5	NS

* These means differ significantly ($p < .02$) from each other.

† The high lambda value of this assay was due to the decreased slope for LH standards, probably a result of LH denaturation prior to assay. Variability in this assay was normal.

‡ Compared to saline controls.

starch gel. The anode itself was directly immersed in a tray filled with 10% sodium chloride.

The gel was run horizontally, at an initial voltage of 165 v for 15 minutes and a final voltage of 350 v for approximately 4-5 hours, for a final distance of the solvent front of 11 cm in a room temperature of 5°C.

The gel was then sliced in half, the top half stained in a nigrosin-naphthalene mixture(2), a calculation made for shrinkage, and the bottom half of the starch gel sliced for testing in the OAAD assay.

Plasma from hypophysectomized cockerels was withdrawn and prepared as reported previously(1). Electrophoresis of a starch gel in which no sample was injected constituted a blank run. If the starch gel preparation was not run, it was covered with hot petroleum jelly and placed in the cold room for the time electrophoresis normally takes. Three drops of 0.1% bovine serum albumin (Pentex) in a slot constituted an albumin run.

Albumin migrated into 2 major bands, a slow-moving and a fast-moving component. On the basis of evidence later thrown into doubt by the OAAD activity of starch gel itself, the slow moving component was determined to be the area of major OAAD activity, and all starch gel blocks were cut out from this region. Starch gel was cut out in 1-cm square blocks, weighing approximately 500 mg, homogenized in saline (3 ml/block), allowed to settle for one hour, and the supernatant suspension withdrawn. All operations were carried out at 5°C. Starch gel supernatant suspensions designated "heated" were placed in boiling water for 30 minutes. All suspensions were refrigerated until the following day when they were injected into the tail vein of immature, pseudopregnant, female rats, 1 ml per 100 g body weight, for measurement in the OAAD assay. The assay procedure was reported previously(1), and the data were statistically analyzed as described(1).

Results. The results are set forth in Table I. Supernatant suspensions from homogenized starch gel blocks had significant OAAD activity, whether the slot contained plasma from hypophysectomized cockerels, albumin, or no sample. The only OAAD results which were not significantly different from saline were obtained when the starch gel preparations were not electrophoretically run.

A highly significant difference between the OAAD activity of starch gel supernatant suspension, placed in boiling water for 30 minutes and untreated aliquots of the same samples, was observed.

Discussion. It is evident that starch gel preparations which have been electrophoretically run cause marked activity in the OAAD assay. Meanwhile, OAAD activity in non-run starch gel preparations was not significantly different from saline. The meaning of this observation is intriguing but obscure. Simple dialysis against distilled water of electrophoretically run starch gel preparations before injection into OAAD assay rats would help clarify the problem. The possible OAAD activity of starch gel in suspension could then be separated from the possible OAAD activity of buffer.

The role which injection of 3 drops of

plasma played in the OAAD activity of starch gel supernatant suspensions appears, by hindsight, to be minimal. Any idea that we were measuring LH was dispelled when placement of starch gel supernatant suspensions in boiling water caused a highly significant increase in OAAD activity. We can offer no explanation for this observation.

In itself, OAAD activity of starch gel preparations following electrophoresis should not mitigate against the use of the OAAD assay. However, a growing list of heterogeneous factors which have OAAD activity, raises questions about the specificity of the OAAD assay itself.

First publications by Parlow on the assay (3,4) reported OAAD activity in the luteinizing hormone (LH)-like materials, human chorionic gonadotrophin and pregnant mare's serum, but no activity in other anterior pituitary hormones, follicle-stimulating hormone, luteotrophic hormone, growth hormone, adrenocorticotrophin and thyrotrophin, except when the dose was large enough to permit ascorbic acid depletion by LH contamination. He found no OAAD response from ether or barbiturate anesthesia, surgical trauma, pitocin, morphine or histamine, but did find OAAD activity in pitressin and epinephrine.

Confirmation of these observations was made by several investigators who reported that there was no OAAD activity in other anterior pituitary hormones(5,6,7), ether anesthesia(5), surgical trauma(5), pitocin(5, 6), and histamine(5,6). Additional factors which had no OAAD activity were acetylcholine, serotonin, salicylate, formalin, insulin, electric shock, and asphyxia(5). Slight OAAD activity was found in high, non-physiological doses of histamine(8) and serotonin (8,9). Hemorrhage and injection of nicotine did not stimulate OAAD activity(6), nor did substance P(8).

Parlow's observation on the OAAD activity of pitressin was confirmed by other investigators(5,6,10). OAAD activity of epinephrine, however, appears to be in doubt(8). To the active list were added norepinephrine(11), lysine vasopressin(6), angiotensin II(10), and arginine vasotocin(1). It should be noted that some of these observations were made on mature, gonadotropin-primed rats(5,6,11), which were relatively less sensitive to LH and more sensitive to pressor substances(6).

A major depleting substance was discovered by McCann in stalk median eminence extracts(8). This substance, designated luteinizing hormone releasing factor (LH-RF), was thought to cause depletion of ovarian ascorbic acid in the immature, pseudopregnant rat by releasing endogenous LH. The OAAD activity in rat stalk median eminence extracts was also found in various other portions of the hypothalamus, although not in cerebral cortex extracts(12). OAAD activity was also found in hypothalami of sheep(10, 13,14,15), and humans(16). Sheep brain cortex did not have significant OAAD activity (13,14). De la Lastra and Croxatto(9), however, found OAAD activity in extracts from human brain, including cerebral cortex, pineal gland and habenular region.

An active depleting factor, which had no LH effect upon the bird itself, appeared in the plasma of hypophysectomized cockerels (1). This OAAD activity increased after fractionation on Sephadex G-100, having activity in 2 major protein fractions; and appeared to have a molecular size larger than is thought to belong to beef LH-RF(17). A similar factor, although of much lower OAAD activity, appeared in the plasma of hypophysectomized rats and was tentatively identified as LH-RF(18). This factor also was observed by Schwartz and Caldarelli(19).

Recently, an additional factor with OAAD activity, has been observed: propylene glycol (9).

It is not surprising that an assay which utilizes a physiological response of obscure meaning(20) should be disturbed by unexpected agents. It is, of course, impossible to search through an infinite number of metabolites for OAAD activity. At the same time, awareness of the possibility that introduction of a new technique or a new physiological state of the animal might render the OAAD assay inoperative, appears to be important for the future investigator.

Summary. Supernatant suspensions from homogenized starch gel used in electrophore-

sis had significant ovarian ascorbic acid depletion (OAAD) activity when injected into immature, pseudopregnant, female rats. Heating of the suspension increased the OAAD activity. The possibility of interference to the OAAD assay for LH by other OAAD-active substances is considered.

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Blood Pressure Response of Adrenal-Enucleated Rats to Restricted And Irregular Feeding Patterns and to Subsequent *ad Libitum* Realimentation.* (30470)

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Within recent years attention has been drawn to peculiar metabolic derangements caused by "stuff-and-starve" regimens as opposed to regular feeding ("nibbling")(2-8). The effect of starvation and subsequent realimentation on the dynamics of the cardiovascular system have also been studied(9-12). It was shown(9-11) that realimentation of previously starving pigs with glucose and starch resulted in hypertension, tachycardia, faltering pulse, extrasystoles and myocardial infarcts. Wilhelmj and McCarthy(11) found that realimentation of normal dogs with carbohydrate and high protein diets resulted in considerable increases of systolic and diastolic blood pressure and heart rate.

In the face of the evidence regarding blood pressure increases on realimentation in intact animals, it was conceivable that restricted and irregular feeding patterns and realimentation could augment blood pressure responses in rats with experimental hypertension. On the other hand, restricted feeding with attendant low sodium intake might suppress increases in blood pressure in a type of hypertension characterized by a large requirement for sodium, namely adrenal-regeneration hypertension. This requirement for sodium in adrenal-regeneration hypertension is particularly true for the first 2 weeks after adrenal enucleation(13,14).

The present note describes experiments on adrenal-enucleated rats to investigate whether restricted feeding patterns during the early post-operative time depressed blood pressure and whether such feeding regimens perma-

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