from or liberated in the gastrointestinal tract (4). An enzyme(secretinase,5,6) which destroys secretin has been described and it is possible that it is present in the liver. Another possibility is that secretin is modified in the liver or stored there and released later. We conclude that the same amount of secretin given into the portal circulation does not have the same effectiveness in inducing pancreatic secretion as when administered into a peripheral vein. Secretin is considered(7) to play a role in neutralization of gastric HCl in the upper small intestine, but the results of our present work indicate that this mechanism may be less effective than hitherto believed.

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Adrenal Cortex and Frog Skin Potentials.* (25161)

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Previous reports demonstrate that in the wake of adrenocortical insufficiency there are alterations in size, shape and conduction velocity of spike potentials in frogs(1), in electrocardiograms(2), and electroencephalograms of humans(3), in electroencephalograms and spinal-cord conduction time and in nerve excitability of rats(4). The present study considers bioelectric potentials of the ventral skins taken from frogs previously subjected to varying concentrations of adrenal cortical substance as effected by adrenalectomy and by administration of whole adrenal cortical extract (ACE) to otherwise normal frogs.

Material and methods. Male 20-40 g frogs, *Rana pipiens,* were divided into 4 groups: *A*) normal, *B*) adrenalectomized, *C*) renal-damaged and *D*) ACE-injected frogs. Operations for adrenalectomy and for renal damage were performed, and the animals used experimentally as previously reported(5). Group *D* was given 0.01 ml ACE (Upjohn) 3 times/week for 4 to 5 weeks. Unpigmented belly skin was removed, rinsed and immersed in 50 ml Ringer at 22 to 28°C. All Ringer solutions were oxygenated and buffered at pH 7.4 with $NaHCO_3$. Thirty-forty minutes after removal, a given skin was placed in a holder similar to that used by Steinbach(6). All potentials were determined to nearest 0.5 millivolt (mV) with a potentiometer and an H-S galvanometer as null apparatus. The electrode half-cell system was calomel-saturated-KCl/saturated-KCl/Ringer-agar-agar/Ringer //skin//, and so on. Two minutes prior to each 10-minute reading, both sides of skin were flushed from a common reservoir with volume of oxygenated Ringer equal to 2 to 3 times that of capacity of reservoir. Skin potentials were followed for 1½ hours; in many instances, longer. The potentials tended to stabilize within *ca.* 60 minutes (Fig. 1).

Results. Results are summarized in Fig. 1, where mean potential values in mV are plotted as functions of time in minutes. Letters and numbers appended to each curve designate the 4 frog groups and number of animals used/group. Mean potential values, with their respective standard errors, for various groups at end of 1 hour are: *A*) -31.2 ± 2.2 mV, *B*)

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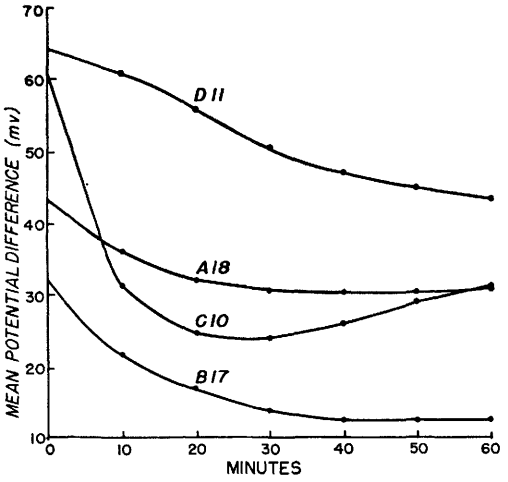

FIG. 1. Mean potential differences in millivolts across isolated ventral skins of common grass frog under experimental conditions indicated by appended letter over each curve: A) control, B) adrenalectomy, C) sham operated, D) injected with ACE, versus time in minutes. Numbers designate number of animals sampled.

-12.5 ± 1.7 mV, *C*) -34.4 ± 4.1 mV and *D*) -43.7 ± 4.6 mV. The following statistical comparisons are based on per cent difference between indicated means with their probability (p) values; $C - A = -0.3\%$ ($p > 0.5$), $B - C = -60.2\%$ ($p < 0.001$) and $D - A = +39.6\%$ ($p < 0.02$).

Discussion. "Normal" skin potentials range from –30 to –50 mV (outside surface is considered electronegative). These results are in agreement with those previously reported (6,7).

In evaluating mean skin potential for adrenalectomized frogs, that for renal-damaged frogs must be considered the control. A comparison of mean skin potentials for "normal" and renal-damaged frogs (*C-A*) shows essentially similar values. The 60% decrease in mean skin potential for adrenalectomized frogs is interpreted as due to adrenocortical insufficiency.

The decrease in skin potentials of adrenalectomized frogs suggests some fundamental change(s) underlying those physicochemical properties normally responsible for its production, *viz.*, metabolism and diffusion(6,7). In support of the metabolic theory are the observations that anoxia and metabolic inhibitors suppress frog skin potentials. Similarly, a decrease in adrenocortical secretions decreases the metabolism of mammals(8), frogs(9) and of certain mammalian tissues(8). It should be noted that anoxia resulting from circulatory failure concomitant with adrenocortical insufficiency(10) may be a contributing factor.

Were diffusive forces wholly or partially involved, there is evidence to suggest that adrenocortical insufficiency increases the permeability of tissue for water(11). This may involve an increase in plasma membrane permeability and/or an alteration in structure of intracellular cement. In support of the former, there is no direct evidence; in support of the latter, some studies indicate that the adrenal cortex has a profound inhibitory effect on substrates (mucopolysaccharides) acted upon by hyaluronidase(12). These substrates may well be the important constituents of the intracellular cement(13).

At the end of one hour the mean skin potential for ACE-injected frogs showed a significant increase when compared to those for normal frogs. Skin potentials for placebo-injected frogs gave results similar to controls; therefore, the action appears to be attributable to injected ACE. One would anticipate that the same mechanism which operated to decrease potentials during adrenocortical insufficiency also served to increase them as a result of an increase in ACE level, but this may not necessarily be the case.

Summary. Potential differences measured across ventral skin of the frog were studied under the influence of varying levels of adrenal cortical hormones. Increased levels of adrenal cortical extract added by injection produced a significant rise in potential difference across the frog skin, while decreased levels of adrenal cortical hormones produced by adrenalectomy significantly reduced the potential difference across the frog skin. Sham operated animals were not significantly different from unoperated controls.

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Spontaneous Hemorrhage Induced by Administration of Aminoacetonitrile and Papain or Anticoagulants to Rats. (25162)

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The principal components of the matrix of epiphysial cartilage are collagen and chondroitin sulfuric acid-protein complex. Certain agents will damage, possibly selectively, each of these constituents. The lathyrogenic agent, aminoacetonitrile (AAN), appears to interfere with polymerization of tropocollagen molecules to collagen fibrils(1); hence, although total collagen content of cartilage from AAN-treated animals is not reduced, the physical properties of such tissue are greatly altered. Intravenous administration of crude papain leads to liberation of chondroitin sulfate from certain tissues, including cartilage (2), possibly as a result of enzymatic destruction of the protein to which the polysaccharide is bound(3). It was thought that the simultaneous effects of the 2 agents, AAN and papain, on cartilage, particularly reparative sequences, might be of interest. During the course of these studies, to be reported elsewhere, widespread, spontaneous subperiosteal hemorrhage has been observed. An exploration of the pathogenesis of this disease state is the subject of this report.

Materials and methods. Albino rats, weighing 45 to 50 g, were used in all experiments. Aminoacetonitrile hydrosulfate, generously supplied by Dr. I. V. Ponseti, was administered by stomach tube in either 1 dose of 20 mg or 2 doses of 10 mg/day. Crude papain was made as a 5% solution in physiological saline and filtered. The usual dose was .5 cc injected into femoral vein. Heparin sodium was administered intravenously, .5 mg being the usual amount. Sodium polyglucose sulfate, generously supplied by Dr. Peter Mora, was administered intravenously, 5 mg being the usual dose. Dicoumarol was added to ground laboratory chow to make a concentration of .1%. Care was taken to cauterize the site of the intravenous injection of papain or anticoagulants lest the animal die of hemorrhage. At autopsy blocks of tissue were fixed in neutral formalin and processed in standard fashion.

Observations. When AAN is administered to normal rats, profound effects are found in rapidly growing epiphysial cartilages in 24 to 48 hours(1). At this time the periosteum may easily be detached from the tibia or calvarium. Gross hemorrhage in these areas is not common at this time, nor is bleeding conspicuous about the ribs, though microscopic subperiosteal hemorrhages are often seen after 2 to 3 days' administration of AAN.

If crude papain is administered intravenously to animals which have received AAN for 1 or 2 days, the rats become listless after 1 to 2 hours. The coat becomes ruffled; in a