is depletion of immune bodies with resulting insufficient antibody for fixing the antigen.

Summary. 1. The administration to mice of Cortisone in doses approximately 50 times the dose for humans produced a striking lymphopenia, loss in body weight, atrophy of thymus and spleen, and diminution in size of adrenal cortex, salivary glands, pituitary, and hibernating fat bodies. A number of histologic changes are briefly described.

2. The anatomic findings correspond to those of the "alarm and adaptation" phenomena of Selye except for atrophy of the adrenal cortex in place of hypertrophy. Cortisone *per se* may produce some of the morphologic changes associated with the "alarm reaction."

3. Administration of these excessively large doses of Cortisone may produce exhaustion of protective mechanisms, including antibody formation.

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Weight Variations of Adrenalectomized Frog Muscles in Different Concentrations of Ringer's Solutions.* (17649)

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It has been shown that following adrenalectomy, body water tends to shift into certain tissues and cells (skeletal muscles of rats(1) and frogs(2), and red cells of dogs(3), cats(4) and rats(5)). According to Winter and Hartman(6), not confirmed by Ponder and Gaunt(7), excised skeletal muscles of adrenalectomized rats shift water in a given hypotonic solution at a greater rate than controls probably because of some alteration in the supposed plasma membranes of the myofibers. Some unrecognized variable, *viz.*, diet, water balance, etc., may be the basis for the observed discrepancy. We reinvestigated(8) the problem studied by the foregoing workers using their experimental technic on skeletal muscles of apparently more favorable material. The frog was selected for the following reasons: 1) nutrition is simplified, assuming that a plentiful supply of testicular fat at autopsy is indicative of at least a "basal" state; 2) imbibition of water is potentially continuous from an aqueous environment(9); 3) isolated muscles of the frog immersed in solutions at room temperature are more within their thermal biokinetic range than are muscles of poikilotherms in solutions from 15 to 20°C lower than their body temperature(10).

Methods. Male *Rana pipiens* (fall and win-

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ter stock) weighing 20 to 30 g were divided into 4 groups (between 15 and 18 frogs/group): 1) *"controlled" adrenalectomized,* 2) *"uncontrolled" adrenalectomized,* 3) *renal damaged* (controls) and 4) *unoperated* (controls) frogs. The methods of dividing the frogs into groups, of adrenalectomy and of determining adrenal insufficiency have been previously discussed(11).

Ringer's solution(12) was prepared in 18-liter lots; the pH was adjusted to 7.4 with $NaHCO_3$. Pyrex beakers, serving individual muscles were filled with 200 cc of one of the following Ringer concentrations: 2X or 3X normal (hypertonic); normal (isosmotic); 75, 50, or 25% of normal strength (hypotonic). The specific muscles studied were the ileofibularis, sartorius and semitendinosus. A pair of specific muscles taken from left and right legs of the same frog give a consistent error for the difference in their individual weights as obtained before and after immersion in a solution of given osmotic strength. Consequently, an equal number of specific muscles excised from an equal number of left and right legs of different frogs were used to produce a mean value (point) on the accompanying graph. Upon excision a muscle was carefully blotted of excessive body fluid, rapidly weighed with a torsion balance, and immersed in a given strength of Ringer's solution at 14 ± 1°C. The time of immersion was recorded from the moment a muscle was placed in solution, and at the end of predetermined intervals (see figures) the muscle was weighed as described (elapsed time *ca.* 20-30 seconds). At the end of an experiment the muscle was returned to aerated normal Ringer's solution at *ca.* 5-7°C. Thereafter, tests were made periodically to determine the duration of survival of its irritability.

The means for any two groups of data were statistically compared. Hereafter, a comparison is considered significant when the difference between a given pair of means exceeds 3 times its probable error.

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Results. Data obtained for any one of the 3 specific muscles following their immersion in Ringer's solution of given osmotic strength are relatively similar. Therefore, ileofibulares from the 4 frog groups immersed respectively in 50%, 2X and in normal Ringer's solution were chosen to summarize the data.

Hypotonic solutions. Data for muscles immersed in 50% Ringer's solution are presented in Fig. 1. An analysis of these curves shows that the rates of increase and the total gain in muscle weights are directly related. Further, the rates of increase and the total gain in weights of muscles from the 4 frog groups following their immersion in hypotonic (50%) Ringer's solution are greater for muscles from "controlled" adrenalectomized frogs than from either unoperated or renal damaged frogs. Similarly, muscles from the latter 2 frog groups give a greater increase in total weights and in rates of weight gain than muscles from "uncontrolled" adrenalectomized frogs. The irritabilities of muscles from unoperated and renal damaged frogs show a longer survival time than muscles from adrenalectomized frogs.

Hypertonic solutions. The rates of decrease and the total loss in weights following immersion of muscles from the 4 frog groups in hypertonic (2X) Ringer's solution are inversely related to the results obtained for the same muscles immersed in hypotonic (50%) Ringer's solution. A statistical analysis of these data shows that decrease in the rates of and the total loss in muscle weights are lowest for "controlled" adrenalectomized frogs, highest for "uncontrolled" adrenalectomized frogs, interposed are renal damaged and unoperated frogs. Incidentally, the latter 2 groups are without statistical significance throughout this report.

Isosmotic solutions. When the percentage changes in mean weights following the immersion of muscles from the 4 frog groups in normal Ringer's solution are compared (Fig. 2), it is found that muscles from the "controlled" adrenalectomized frogs and from unoperated and renal damaged frogs increase their weights; while muscles of "uncontrolled" adrenalectomized frogs lose weight.

Discussion. Weight changes of skeletal

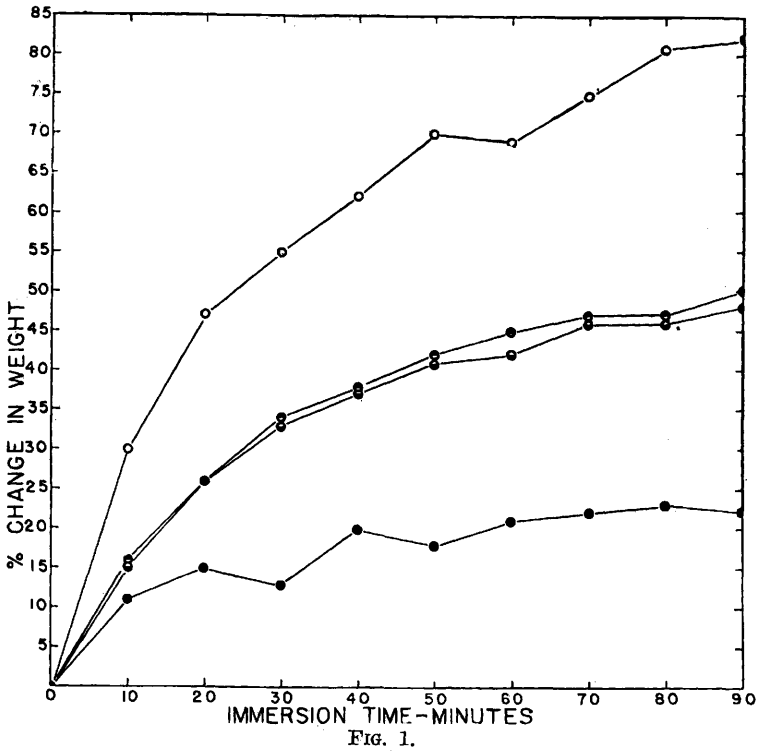

FIG. 1.

The percentage change in the mean weights of ileofibularis muscles excised from the following designated groups of frogs immersed in 50% Ringer's solution (ordinates) for varying periods of time in minutes (abscissae). Frog groups: "controlled" (open circle) and "uncontrolled" (solid circle), adrenalectomized frogs; and the unoperated (upper half solid circle) and renal damaged (lower half solid circle) frogs. Temp. 14 ± 1°C; pH 7.4.

muscles in solutions of various osmotic strengths indicate that they are not perfect osmometers. The evidence suggests "that the osmotic behavior is a reflection of forces relating to ion accumulating mechanisms and is not due to the existence of a discrete semipermeable membrane of fixed dimensions" (13). Until these forces are understood for the apparent osmotic behavior of "normal" muscles, it seems inadvisable to venture an interpretation of the osmotic behavior of muscles of adrenalectomized animals.

Muscles from "controlled" adrenalectomized frogs show higher rates of water shift on immersion in a given hypotonic solution than do muscles obtained from either renal damaged or unoperated frogs, (Fig. 1). These results parallel those of Winter and Hartman(6) for adrenalectomized rats. They differ in that the total amount of water shifted is a function and not independent of the experimental group from which the muscle is obtained. On the other hand, muscles from "uncontrolled" adrenalectomized frogs show lower rates of water shift and the total water shifted is less than for muscles from either renal damaged or unoperated frogs. These results simulate those of Ponder and Gaunt(7). The crucial variable accounting for the apparently divergent results obtained for the 2 groups of adrenalectomized frogs as well as for the two reports on adrenalectomized rats(6,7) is the difference in water load(14). It has been established that certain cells(3,4,5) and, more pertinently, skeletal

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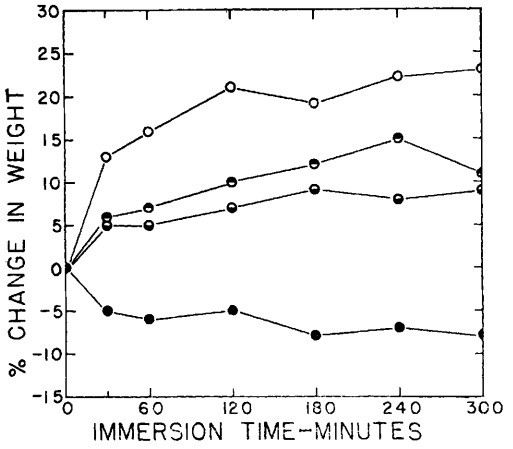

FIG. 2.
The percentage change in the mean weights of ileofibularis muscles excised from the following designated groups of frogs immersed in normal Ringer (ordinates) for varying periods of time in minutes (abscissae). Frog groups: "controlled" (open circle) and "uncontrolled" (solid circle) adrenalectomized frogs; and the unoperated (upper half solid circle) and renal damaged (lower half solid circle) frogs. Temp. 14 ± 1°C; pH 7.4.

muscles of rats(7,1) as well as of frogs(2) accrue a water load following adrenalectomy. The effect of the initial water load on weight change is dramatically shown when muscles are immersed in normal Ringer's solution. All muscles of the 4 frog groups behave "normally" in that they increase their weights(15) (*i.e.*, solution acts hypotonically), except for muscles of "uncontrolled" adrenalectomized frogs which decrease their weights (*i.e.*, solution acts hypertonically). These results on "uncontrolled" adrenalectomized frogs are in accord with those of Ponder and Gaunt on adrenalectomized rats, with one exception: although their data showed no statistical significance for weight changes in hypotonic solutions the present data do. This apparent inconsistency is probably explainable on the basis of the kind of animal employed. The frog given a copious aqueous environment continuously imbibes water through its integument(9), and in adrenal insufficiency increases its body (water) weight(16,14), whereas the rat is dependent on the physiologically little understood "drive" called thirst to aid in maintenance of body water, and in adrenal insufficiency decreases its body weight(17).

That the irritability of skeletal muscles of adrenalectomized frogs fail to survive as long as muscles from either renal damaged or unoperated frogs is to be anticipated from studies of direct stimulation of frog muscle with respect to isotonic contractions and work performance(16,18).

Summary. Ileofibularis, sartorius and semitendinosus muscles were obtained from 4 groups of grass frogs: 1) *"controlled" adrenalectomized;* 2) *"uncontrolled" adrenalectomized;* 3) *renal damaged* (controls); and 4) *unoperated* (controls) frogs. These muscles were studied with regard to changes in their initial weights following predetermined periods of immersion either in hypotonic, isosmotic or in hypertonic Ringer's solution. The rates of increase and the total gain in weights of muscles from the 4 frog groups following the immersion of the muscles in hypotonic (50%) Ringer's solution are highest for the "controlled adrenalectomized frogs, lowest for the "uncontrolled" adrenalectomized frogs. The same muscle groups when studied in hypertonic (2X) Ringer's solution give results inversely related to the foregoing values. In normal Ringer's solution, muscles from the "uncontrolled" adrenalectomized frogs lose water, those from "controlled" adrenalectomized, renal damaged and unoperated frogs behave normally by increasing their weights. These results are interpreted on the basis of the difference in the initial water loads of the skeletal muscle of the respective groups of frogs which are known to exist prior to experimental use.

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