360 per minute.

The almost complete absence of sclerotic changes in the group of animals treated with epinephrine and desoxycorticosterone is difficult to explain. Further, the Selye group(9), working on chicks and rats, obtained hypertension and vascular changes with the use of desoxycorticosterone. The well known action of desoxycorticosterone in the regulation of cell membrane permeability and its effect on electrolyte balance may be involved in facilitating detoxication in a state of hypoxic metabolism. When thyroxin is added to this treatment desoxycorticosterone failed completely to inhibit the sclerotic changes. It is possible that the permeability changes due to desoxycorticosterone are no longer sufficient to compensate for the enormous catabolic injury to the tissues of the vessels.

Summary. Thyroxin and epinephrine treatment in rabbits was shown to produce aortic sclerosis in a greater percentage of cases, and a more severe grade of such sclerosis, than epinephrine alone. Desoxycorticosterone decreases the amount of sclerosis due to epinephrine alone but failed to influence the sclerosis due to the combined epinephrine-thyroxin treatment.

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Effect of Cortisone Administration upon Nucleic Acid Composition of Rabbit Liver.* (19231)

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Though purines appear in increased amounts in urine of both animals and humans treated with cortisone or ACTH(1-4), it is not clear whether the uricaciduria is merely a reflection of decreased tubular reabsorption of uric acid(1,4), or results partly from lymphoid tissue destruction, since this atrophies following the administration of ACTH(5-7). A portion of the pentose nucleic acid (PNA) of hepatic cells of animals is labile and can be reduced by chemical injury(8,9) and starvation(9-11), and returns readily during recovery from either type of stress. Because of these observations, experiments were planned to demonstrate whether cortisone could reduce the amount of PNA in the liver.

Plan of experiments. Cortisone was injected intramuscularly in daily doses of 25 mg for 1, 2, 3, and 6 days in 10 albino rabbits, 1-2 kg in weight. The animals were sacrificed 24 hours after the last dose of cortisone. In addition, 2 animals received cortisone for 3 days and were sacrificed 9 days later. Six animals served as controls. The animals were stunned by a blow on the head, then exsanguinated by severing the hepatic vein. The livers were dissected free and weighed after removal of the gallbladder. A 2-4 g portion of the liver was weighed wet, then dried first over a water bath, then in an oven at 100°C to constant weight. A second portion (2-4 g)

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TABLE I. Effect of Parenteral Administration of Cortisone on Composition of Rabbit Livers.

Animal No.	Days cortisone	% dry wt	mg N/g wet wt	μg DNA/g wet wt	μg PNA/g wet wt	PNA/DNA
59	0	—	30.60	616	2685	4.36
				645	2830	4.39
1	0	31.34	35.70	807	2692	3.34
				789	2847	3.61
2	0	29.44	35.70	786	2987	3.80
				821	3163	3.85
3	0	31.64	38.10	817	3543	4.34
				884	3899	4.41
4	0	32.80	36.55	764	3495	4.57
				772	3418	4.43
203	0	29.50	29.07	541	2670	4.94
				569	2871	5.05
Avg & S.D.	0	30.94 ± .38	34.21 ± 4	734 ± 108.42	3091 ± 394.8	4.26 ± 1.49
99	1	30.90	22.93	382	2056	5.38
				401	1956	4.88
100	1	29.58	24.15	486	2098	4.32
				513	2058	4.01
Avg	1	30.24	23.54	446	2042	4.65
101	2	31.05	21.40	284	1830	6.44
				287	1680	5.85
102	2	32.09	20.12	356	2306	6.47
				356	2130	5.98
Avg	2	31.57	20.76	321	1987	6.19
97	3	30.98	20.70	294	2148	7.31
				305	1990	6.52
98	3	31.21	16.30	227	1556	6.85
				227	1734	7.64
199	3	28.50	17.75	300	2631	8.77
				300	2676	8.92
200	3	29.40	20.50	396	2600	6.57
				358	2485	6.94
Avg	3	30.02	18.81	301	2228	7.44
43	6		20.30	310	2489	8.03
				323	2571	7.96
44	6		17.00	231	2103	9.10
				257	1972	7.67
Avg	6		18.70	280	2284	8.19
201*	3	27.90	23.40	433	2390	5.52
				433	2537	5.86
202*	3	26.30	31.20	564	3514	6.23
				498	2463	4.95
Avg	3	27.10	27.80	482	2726	5.64

* These animals received cortisone for 3 days but were not killed for 9 days thereafter.

was expressed through a meat press onto a torsion balance pan, weighed to .01 g and then quantitatively transferred to a Potter-Elvehjem glass homogenizer. The homogenate was diluted with slightly alkaline 0.85% NaCl so that each ml of solution contained 0.1 g of wet liver and portions thereof were used for all chemical analyses. Two 2 ml fractions of the brei were used for nucleic acid analyses. This was thoroughly mixed with 5 ml of trichloroacetic acid (TCA), kept at 10°C for one hour, centrifuged, and the precipitate washed once with 5 ml of 5% TCA. The supernatants were pooled and subsequently analyzed for DNA (desoxypentose nucleic acid) and PNA. Since under the conditions of the experiments there was no difference in the supernatants in the values for DNA and PNA between the controls and the cortisone-injected animals, these results will not be tabulated. The sediment was extracted with 5 ml of 5% TCA at 80°C for 30 minutes, centrifuged, re-extracted once more with 5 ml of 5% TCA for 5 minutes at 80°C, and recen-

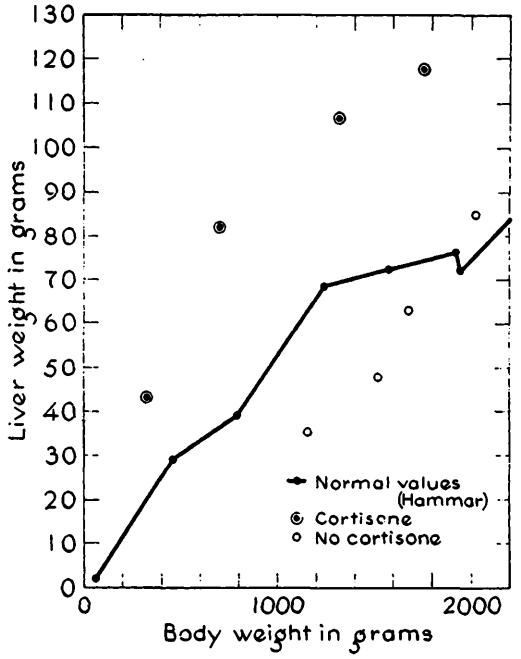

FIG. 1. Relationship between body wt and liver wt. Wt for 4 normal and 4 cortisone treated animals from the present experiment are recorded. Also included are normal values obtained from Hammar(21) from a much larger series of rabbits.

trifuged. These supernatants were pooled and analyzed for DNA and PNA in duplicate. PNA was determined by the orcinol reaction; DNA by diphenylamine color; and nitrogen by a micro Kjeldahl procedure in duplicate on 1 cc of 1:10 brei. These have been described previously(12). One ml of concentrated HCl was added to 9 ml of the original brei. This was then placed in boiling water for 10 minutes. After centrifuging, reducing substance in the supernatant was determined by the Somogyi method(13,14). When kidneys were analyzed for PNA, DNA, and N, they were treated in the same fashion as the livers. Tissues for histological examination were obtained immediately after the whole liver was weighed. These were placed in Lavdowsky's solution(15), and then examined with the following stains: Feulgen (DNA)(16), Ribonuclease-Toluidine blue (PNA)(16,17), periodic acid, Schiff's (glycogen)(18), Ponceau-2R (acidophilic protein)(19), Sudan III (lipid), and Hematoxylin-Eosin.

Results. Table I summarizes the days of cortisone administration, changes in wet and dry weight, nitrogen, PNA,† DNA,† and the ratio PNA/DNA. The following may be noted: The per cent of dry weight did not change appreciably, even though total liver weight was apparently increased (Fig. 1). The total N per g of wet weight decreased steadily with increasing time of cortisone administration. This was also true of the DNA. The concentration of PNA decreased only for the first two days and then remained almost the same thereafter. Because of the difference between the behavior of the DNA and PNA, the ratio of PNA/DNA became progressively greater. The livers of the two animals that received cortisone for 3 days only and were subsequently sacrificed 9 days later, showed results which were intermediate between those of the normal animals and those treated for 3 days, except for the per cent dry weight. This had decreased appreciably, suggesting some edema of the liver.

Table II indicates that there was a considerable increase in reducing substance, presumably glycogen, in the liver of the animals in which this was determined.

The nucleic acid composition of the kidney was determined in 4 animals before the administration of cortisone and in 2 animals after administration of cortisone for 3 days. In the untreated animals the average value of PNA was 1670 μg/g and for DNA 1246 μg/g, ratio PNA/DNA being 1.34. After cortisone the PNA was 1715 μg/g and DNA 1131 μg/g, while the ratio was 1.51 Composition of the kidney was not significantly altered by this type of treatment. In the 2 animals that had received cortisone, the livers were analyzed and the characteristic alterations anticipated were observed. It is obvious, therefore, that while the liver is significantly affected by cortisone administration, the kidney is not.

The histochemical observations are summarized in Table III and illustrated in Fig. 2 to 5. They confirm the chemical observa-

† The notations DNA and PNA when used in the text refer to total nucleic acid while in the tables indicate only the carbohydrate moieties, since these were the components measured. mg Desoxyribose x 2.3 = mg DNA. mg Ribose x 2.14 = mg PNA.

TABLE II. Carbohydrate Content of Rabbit Liver.

	Animal wt, kg	Liver wt, g	% CHO in liver	Total CHO in liver, g	Liver CHO / Body wt × 1000
No cortisone	2.08	84.5	2.09	1.77	.85
	1.18	36	3.25	1.17	.992
Cortisone 3 days	1.26	107.5	5.32	5.72	4.54
	1.76	118	4.65	5.49	3.12
	.750	83	3.04	2.52	3.36

TABLE III. Histochemical Examination of Rabbit Livers.

	Normal	Cortisone	Cortisone-recovery
Acidophylic protein (Ponceau 2-R)	4*	1*	3*
Glycogen (PA-S)	2*	4*	3*
PNA (toluidine blue pH 7)	4*	1*	3*
DNA (Feulgen)	1*	1*	1*
Sudanophilic lipid	1*	1*	1*

* The intensity of staining has been graded from 1 to 4*.

tions and further indicate that during the relatively short period of cortisone administration there was no striking increase in fat in the liver.

Discussion. Though sufficient data are lacking to select the proper explanation for changes in liver composition, several of these deserve mention. A first possibility is as follows: Under the influence of cortisone two phenomena occurred. (1) liver cells increased in size (as the microscopic sections indicate) and (2) there was an absolute increase in the amount of PNA. These two effects provided (a) that the increase in cell size was relatively greater than the increase in PNA, and (b) that the DNA remained constant per cell, would decrease per g of liver of the PNA and raise the ratio of PNA/DNA. There is evidence for and against this explanation. That there could have been a two-fold increase in the PNA seems doubtful from the evidence furnished by Skipper *et al.*(20). They observed that cortisone actually inhibited the incorporation of C^{14} labeled formate by both DNA and PNA in the viscera of mice. Hence, synthesis of new PNA under the present experimental conditions seems unlikely. That there was an absolute increase in the size of the livers of the cortisone-treated rabbits is suggested by Fig. 1. No evidence can be advanced to indicate that liver weights had increased sufficiently to provide the observed alteration in the concentration of PNA and DNA, for it was obviously impossible to weigh the livers before and after cortisone. But even if such data were available these could not explain the change in the ratio PNA/DNA. Because of these objections, this explanation for the observed changes cannot be entirely acceptable.

The second explanation which can be advanced is that there was a decrease in both PNA and DNA per cell, but the decrease in DNA was greater than that of PNA. To attempt to support this hypothesis, information was sought from histochemical observations. Evidence was required as to whether PNA actually had increased or decreased in each cell. Examination of the sections stained with toluidine blue indicates that in the cortisone-treated animals, there was a tremendous decrease in the ribonuclease-hydrolyzable basophilia (PNA) per cell (Fig. 3, 5). Since the PNA seemed to decrease in each cell, the alterations in PNA/DNA ratio could only have occurred if there had been a concomitant decrease in the DNA which was greater in amount than the decrease in PNA. It has always been assumed that DNA-histone when once formed was insoluble in the living cell (22), only cell death resulting in its removal. Were this correct, then the results indicated by this experiment would be at least somewhat anomalous. This is particularly true since there was no evidence of cell necrosis following cortisone administration though serial sectioning of the livers was not performed. How-

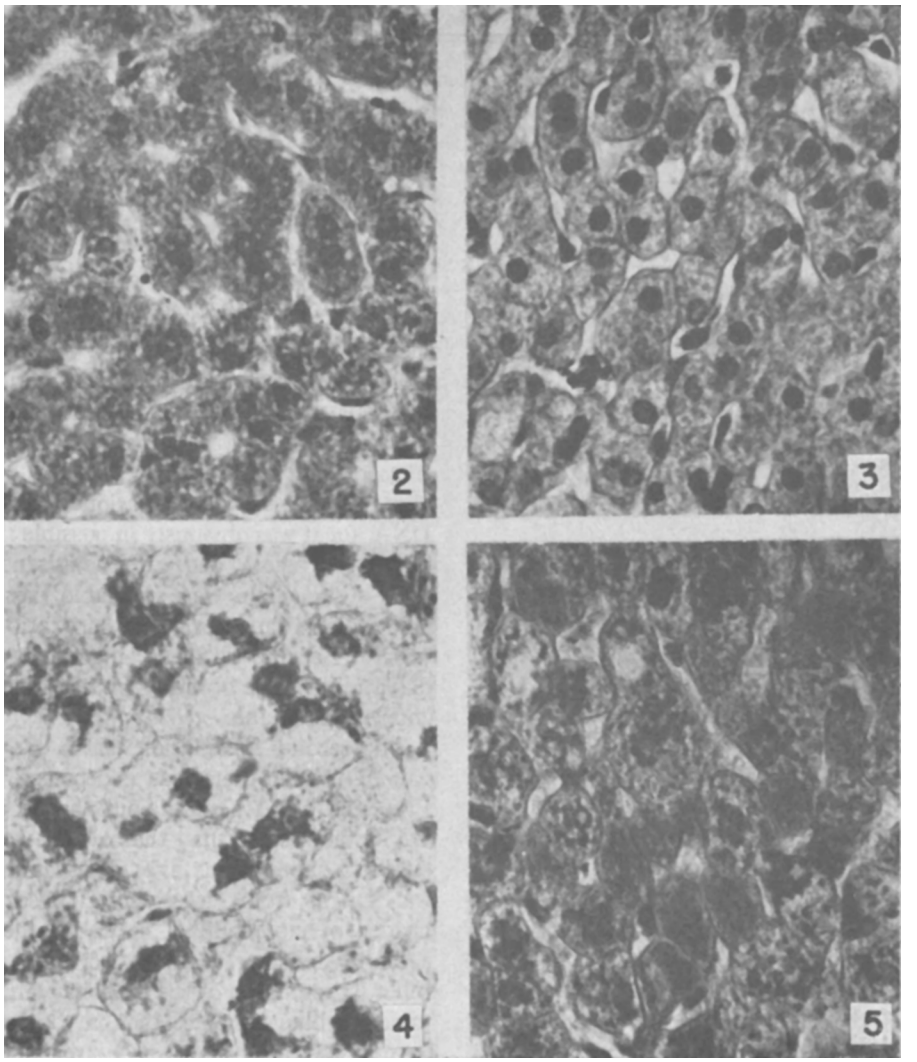

FIG. 2-5. 10 μ sections of rabbit livers stained with .06% toluidine blue at pH 7 for 25 min. Sections in Fig. 2, 4 and 5 were hydrolyzed in Clark-Lubs' buffer, pH 6.8 at 37°C for 5 hr prior to staining. Section in Fig. 4 was hydrolyzed under identical conditions in buffer containing 1 mg of ribonuclease per ml. 500 ×.

FIG. 2. Normal rabbit. Note the abundant, granular cytoplasmic basophilia.

FIG. 3. Section of same liver as above. Absence of granular cytoplasmic basophilia following ribonuclease-hydrolysis.

FIG. 4. Six days of cortisone-administration (animal 43). Cytoplasmic enlargement, and absence of basophilia except in perinuclear area.

FIG. 5. Cortisone-administration for 3 days followed by 9 days' "recovery" (animal 201). Note partial repletion of cytoplasmic basophilia.

ever, Germuth *et al.*(23) have reported that necrosis does occur in the livers of rabbits treated for 18 days with 10 mg of cortisone per day. Schneider and Hogeboom suggest that at least 60% of the DNA in the resting liver cell nucleus is soluble(24). Furthermore, Putnam and Kozloff(25) from studies of bacteriophage production by bacteria suggested that only 30% of the bacterial DNA was genetically important. The remainder might be the soluble DNA of Schneider and Hogeboom, and also that portion which was released from the livers of rabbits to whom cortisone had been administered. This second

explanation depends for its justification largely upon the staining characteristics of PNA. It is possible that under the altered conditions which obtain in a liver cell following the administration of large doses of cortisone, there would be interference with the normal affinity of PNA for toluidine blue. Therefore, this explanation also cannot be accepted without reservation.

A third explanation might be advanced. The change in concentration of DNA might be due to destruction of nonhepatic cells, particularly lymphocytes. The disappearance of these cells in conjunction with an increase in hepatic cell size would yield analytical results of the sort obtained. This concept would receive substantial support if it were known that lymphocyte cytoplasm contained less PNA per cell than did liver cells, and if it were possible to demonstrate a significant difference in the number of lymphocytes between the normal and cortisone-treated animals. A careful search of the normal rabbit livers failed to demonstrate any significant number of lymphocytes, hence cortisone treatment could produce no decrease. However, serial sections would be necessary to establish this point beyond question.

It is known that cortisone causes enhancement of infection by certain bacteria(26) and viruses(27). Although there was no evidence of infection in the rabbits used in these experiments, the possibility that the biochemical alteration in the liver may have been due to injury by an undetected infectious agent cannot be excluded. A partial solution to the dilemma posed by these observations may come from the technic of counting nuclei and determining the quantity of DNA and PNA per nucleus. By this means it should be possible to ascertain whether these actually are altered in each living cell and, if so, the extent of this change.

Summary and conclusions. Livers of rabbits that received 25 mg of cortisone for 1, 2, 3, and 6 days were studied chemically and histologically. Under this stimulus, nitrogen content decreased and carbohydrate content increased in the livers. The PNA and DNA per g of liver both decreased but the ratio of PNA/DNA rose steadily with increasing number of days of cortisone administration. Analysis of livers of 2 animals killed 9 days after cessation of cortisone injection indicated repletion of DNA, PNA, and nitrogen. The nucleic acid composition of the kidneys was not altered by cortisone. A number of alternate explanations for the observations are advanced.

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Antithyroxine Properties of *N*-(2,5 Dihydroxyphenyl) Pyridinium Acetate.* (19232)

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(Introduced by Joseph L. Lilienthal, Jr.)

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With the increasing evidence that the circulating thyroid hormone is free thyroxine(1-3) an added importance is afforded recent studies (4-6) directed at determining agents which may possibly inhibit the action of thyroxine. Such research may elucidate the little understood mechanisms whereby the thyroid hormone acts to stimulate the metabolism of body cells as well as to regulate thyrotropin production by the anterior hypophysis. As Harington(7) has indicated, structural analogues of thyroxine possessing properties metabolically competitive to thyroxine are of physiological interest and potential clinical application. The observations by Woolley(4) that several structural analogues of thyroxine inhibit thyroxine-induced metamorphosis of tadpoles have been confirmed and extended by Williams *et al.*(8), by Frieden and Winzler (9.10). and in our laboratory(11). However, several investigators(8,11-13) have been unable to demonstrate that these analogues inhibit to any noteworthy degree the effect of thyroxine when tested in animals of higher forms.

In 1948, while the tadpole metamorphosis test was being used as a screening method to determine the antithyroxine potentialities of a large number of compounds, it was found in this laboratory that *n*-(2,5-dihydroxyphenyl) pyridinium acetate† (DHPPA) appeared to have such properties. Subsequently, the effects of this compound on the oxygen consumption of normal rats and of normal rats receiving thyroxine were observed. These studies comprise this report.

Methods and results. A. *Metamorphosis of tadpoles. Xenopus laevis* tadpoles, bred and raised according to the method of Deansley and Parkes(14), were kept in "livered" tap water‡ and fed a mixture of dried powdered liver and flour until they reached 18-26 mm in length. The tadpoles were transferred to "livered" water in beakers of 250 ml capacity, 5 tadpoles to each beaker; graded doses of thyroxine and/or DHPPA were added, and the final volume of each beaker was brought up to 200 ml. All beakers were placed in a water bath at room temperature. At the end of the third day the tadpoles in each beaker, after a brief rinsing to remove any residual drug, were transferred into clean beakers, each containing 200 ml of "livered" water. The beakers were returned to the water bath until the seventh day when the test was terminated. The animals received no food during the experimental period. Each tadpole was observed daily for evidence of metamorphosis.

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‡ "Livered" tap water was prepared by adding 100 mg dried powdered liver to each gallon of tap water and then filtering after 24 hours. This procedure removes excess chlorine.