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Keyphrases

Acetarsone—acid-base chemistry
Arsthinol—acid-base chemistry
Ionization constants—acetarsone, arsthinol
Acid-base titration—acetarsone, arsthinol
Iodometric titration—acetarsone, arsthinol
UV spectrophotometry—analysis

Hypoglycemic Activity and Chemical Structure of the Salicylates

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The results obtained in a study undertaken to clarify both the structural requirements of salicylate hypoglycemic activity in the hope of diminishing the unpredictability associated with it and the mechanism of such activity are presented in an effort to shed further light on the pathophysiology of diabetes mellitus. Results of the structure studies point to the carboxyl group as being essential to the salicylate hypoglycemic activity, the hydroxyl radical in the *ortho* position as being beneficial but not imperative with respect to this activity. Results of the action mechanism studies involve suppression of the release of fatty acids from adipose tissues in salicylate hypoglycemic activity.

THE SALICYLATES have been used for nearly a century in the treatment of diabetes mellitus but their usefulness as hypoglycemic agents has been limited by the fact that the doses required to bring about a significant blood glucose lowering response are relatively large and the response is somewhat unpredictable in that a wide variety of effects other than the hypoglycemic effect is elicited concomitantly. The advent of insulin and the oral hypoglycemics signaled a loss of interest in the antidiabetic action of these compounds.

The effectiveness of salicylates in decreasing glycosuria and hyperglycemia in various animal preparations (1–6) and in man (7–12) is, however, well established. The discovery of the cause of their unpredictability and the mechanism of their action has not been achieved.

Salicylates diminish the hyperglycemia of some, not all, human diabetics, both of maturity-onset and juvenile types. Salicylate-induced hypoglycemia can be demonstrated in alloxan or pancreatectomized diabetic animals of different species.

In general, a fairly large dose of salicylates is required to produce significant lowering of blood sugar. In man the maximal hypoglycemic activity is reached at plasma salicylate concentrations of between 20 and 30 mg./100 ml. Salicylate levels as low as 6 to 10 mg./100 ml. have been claimed to reduce hyperglycemia effectively (13–16). The effect of salicylates to reduce hyperglycemia in the rat is not abolished by hypophysectomy (6, 17) or by bilateral adrenalectomy (5, 18).

The blood sugar response to salicylates in humans and animals is related to blood salicylate level and to initial blood glucose concentration. The hypoglycemic activity of salicylates is more

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pronounced when the hyperglycemia of diabetes is marked and is more pronounced when fasting rather than postprandial blood levels are involved. It would appear that salicylates do not have a significant hypoglycemic effect in normal (euglycemic) man or animals. On the other hand, many salicylate poisoning cases have been reported to be complicated by hyperglycemia and glycosuria (19, 20).

The study reported was undertaken to determine the structural requirements of the hypoglycemic activity of the salicylates and the mechanism of this activity.

EXPERIMENTAL

The animals used were alloxan-diabetic male and female rats, weighing between 130 and 150 g., which were the offspring of Carworth Wistar males and Charles River females. The rats selected for the production of diabetic animals were starved for 24 hr. but were given water *ad libitum* during this period. Glucose determinations on blood samples withdrawn from the stubs of the cut tails of the rat just prior to their alloxan injections served as control values. Each rat was given a subcutaneous injection of the first alloxan dose of 150 mg./kg. body weight. Two days later, each rat received a subcutaneous injection of the second dose of the diabetogenic drug of 120 mg./kg. The incidence and severity of the resultant diabetes could be judged by the appearance of characteristic symptoms and the subsequent blood glucose determination. Sometimes it was necessary to give a third and even a fourth dose of the drug of 100 mg./kg. each at 2-week intervals until the diabetic symptoms became very apparent and the fasting blood glucose was more than 200 mg.%.

Most diabetic rats could survive for at least 1 year without any insulin treatment, provided good care was maintained. Adequate food and water, optimal room temperature, and clean cages were indispensable. Usually the diabetic rats developed the characteristic "poly triad" shortly after the first two injections. Retinopathy was recognizable within 1 to 2 months after alloxan treatment. Retardation of growth was easily observed in a comparison of the injected animals with their normal littermates. The most reliable criterion of diabetes was, of course, the lowered glucose tolerance with the pronounced hyperglycemia.

The diabetic rats were not used in the experiments of this study unless they had survived for at least 3 months after the alloxan treatment. A diabetic rat would be used repeatedly for many experiments, but it was required to have a 1-month period of rest after each experiment to ascertain the clearance of any residual drug effect.

Screening of salicylate derivatives and compounds similar in structure to salicylate for their effects on the blood sugar levels of the rats was carried out as follows.

The food but not the water intake of normal as well as diabetic rats was restricted for 18 to 20 hr., in order to establish a rather stable fasting blood glucose level. Blood samples from tail stumps were taken before the rats received any treatment. Then the animals were given the test compound of specified dosage by subcutaneous injection. If the compound could not be made soluble enough to prepare an aqueous solution for injection, it was administered orally to the animal in a freshly-prepared CMC medium in accord with a modified Dulin's formula (benzyl alcohol omitted); sodium carboxymethylcellulose, 0.5%; polysorbate 80,[1] 0.4%; sodium chloride, 0.9%. blood samples were collected 0.5, 1, 2, 3, 5, and in most cases 7 hr. after injection or 1, 2, 3, and 5 hr. after oral administration.

Nondrug control tests were accomplished with normal and diabetic rats receiving either saline injection or oral administration of CMC medium only. In most experiments one diabetic rat receiving a subcutaneous injection of 4 mmoles of sodium salicylate/kg. body weight served as the drug control. Furthermore, glucose tolerance tests for a few normal and a few diabetic rats were performed and served as a reference.

The maximal dose level was 4 mmoles/kg. body weight. If a toxic reaction were observed at such a dose, lower levels were employed until there was no evidence of toxicity. The minimal dose, 0.5 mmole/kg., was reached in some instances. The limit for the volume of the injection or oral dose was fixed at 5 ml./kg. body weight. The pH of the injected solution was adjusted to the range between 6.5 and 8.5.

It was found important to handle the animals as gently as possible and to avoid any unnecessary stress and injury. If the animal were too feeble, its response to drug was not considered reliable. The animals undergoing testing had to be able to tolerate the 18-hr. fast plus the 7-hr. test period. Care was taken to prevent coprophagy during the fast. Test rats were not used more frequently than once each month.

The blood glucose concentration of the samples was determined by the enzymatic method described by Saifer and Gerstenfeld (21) and Washko and Rice (22) slightly modified to conserve the blood of the animal.

In this method, when the animal was immobilized, 50 μl. of blood was taken from the stump of the severed tail tip by means of a lambda pipet and diluted to 1 ml. with distilled water. To this 1 ml. (20-fold dilution), 0.5 ml. each of barium hydroxide solution and zinc sulfate solution were added with thorough mixing to precipitate the proteins. The now 40-fold diluted specimen was centrifuged and 0.5 ml. of the clear supernatant liquid was pipeted to a test tube. To the tube containing this blood specimen 4.5 ml. of Glucostat reagent solution was added. The test tube was incubated in a water bath at 37° for at least 30 min. At the end of incubation 2 ml. of 17.1 N H_2SO_4 was added to develop a pink color. The color intensity was measured by the absorbance of the sample at 540 mμ in a spectrophotometer (Coleman). Water, instead of blood, processed in the same way, served as a blank.

A standard curve was prepared during each assay with standards of known amounts of glucose, as was the case in the different dilutions of the glucose stock solution. The concentration of glucose in a specific

[1] Tween 80, Altas Chemical Industries, Inc., Wilmington, Del.

blood sample in mg./100 ml. of blood could thus be read directly by converting its absorbance to the standard curve glucose equivalence.

The Novak colorimetric ultramicro method (23), slightly modified, was used for the determination of plasma nonesterified fatty acids (NEFA) in samples.

In this method, 50 μl. of blood was taken from the stump of the severed tail tip and mixed immediately with 75 μl. of heparinized saline. The blood suspension was centrifuged and an aliquot of 70 μl. of plasma solution was taken and kept frozen until the start of the assay. To the frozen plasma solution 0.4 ml. of Dole's extraction mixture (24) was added. The liquids were mixed by vibration and the test tube was cooled in an ice bath. After 0.3 ml. of heptane and 0.5 ml. of water were added, the contents of the tube were thoroughly mixed again. Three-tenths milliliter was drawn from the upper heptane phase and transferred to another test tube. To it 0.5 ml. of chloroform–heptane mixture and 0.6 ml. of the cobalt reagent were added. The solution was thoroughly mixed and centrifuged. Six-hundredths milliliter of the upper chloroform–heptane layer was transferred to a third test tube containing 0.75 ml. of the indicator solution and an orange color developed. The color intensity was measured by the absorbance of the sample at 500 mμ (Coleman spectrophotometer). A tube which contained heparinized saline instead of plasma solution was processed in the same way to serve as a blank.

A standard curve was prepared in each assay by measuring the absorbance of standard palmitic acid solutions containing known quantities of the fatty acid. The concentration of NEFA in the assay sample in meq./l. of plasma could be read directly from the corresponding absorbance on the standard curve.

RESULTS AND DISCUSSION

The effect of salicylic acid on the blood glucose of alloxan diabetic rats is illustrated in Fig. 1. The effect of each of the compounds studied on the blood sugar level of the diabetic rat is listed in Table I.

Chemical Structure–Biological Activity Relationships—Acetylsalicylic acid possessed almost the same hypoglycemic activity as salicylic acid. Salicylamide *elevated* blood sugar levels.

Block of carboxyl group, as in semicarbazone and hydrazine derivatives, caused abolition of the glucose lowering action of salicylic acid. Benzoic acid was less effective than salicylic acid as an hypoglycemic agent, yet it had significant hypoglycemic activity.

Insofar as the hydroxyl group is concerned, phenol had no significant activity on blood sugar concentration. Catechol also had no activity. Both were toxic. An additional hydroxyl radical on the benzene ring of salicylic acid eliminated hypoglycemic activity. If the hydroxyl group were replaced by an amino group, such as anthranilic acid, the hypoglycemic effect was reduced but was still significant. If it were substituted by a nitro or mercapto group or by fluorine, blood sugar tended to rise and toxicity was increased.

If the benzene ring were alkylated, the toxicity was increased and the hypoglycemic activity disappeared. The toxicity of alkylated salicylic acid was increased in proportion to the number of carbon

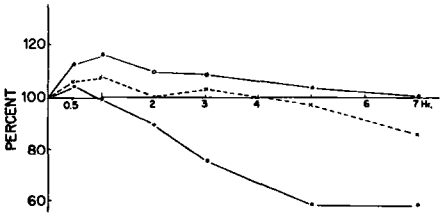

Fig. 1—Effect of salicylic acid (S.A.) on blood glucose of diabetic rats. Key: ○, 11 rats, 86.4–444.0 mg./100 ml., 5 ml. saline/kg., s.c.; ×, 5 rats, 130.0–193.6 mg./100 ml., 2 mmoles S.A./kg., s.c.; ●, 7 rats, 88.0–375.0 mg./100 ml., 4 mmoles S.A./kg., s.c.

atoms of the alkyl group or groups. Also, the position of alkylation made no difference. If the benzene ring were replaced by a naphthalene ring, the effect on blood glucose was like that of alkylation. Replacement of the benzene ring with a 5-membered ring seemed to increase hypoglycemic potency.

Nicotinic acid analogs had greater hypoglycemic activity than salicylic acid itself. The sugar-lowering activity of nicotinic acid and 2-hydroxynicotinic acid was even more pronounced. Isonicotinic acid derivatives did not follow this pattern. Pyrazine and pyrimidine analogs of salicylic acid had little effect to alter blood sugar level.

In summary, the carboxyl group of salicylic acid appeared to be responsible for the hypoglycemic activity. The hydroxyl group at the *ortho* position was not imperative but beneficial to the hypoglycemic action.

Mechanism of Action of Salicylates—A considerable difference of opinion exists on the matter of the mechanism of salicylic acid's hypoglycemic effect, some investigators being of the opinion that these agents suppress gluconeogenesis, some that they promote glycogen formation. In addition, a number of other mechanisms representing possibilities for hypoglycemic activity have been introduced. None is supported by available evidence. Salicylates do not diminish the *in vivo* rate of intestinal absorption of glucose in either animal preparations (25, 26) or in man (10, 27). Also, they do not reduce dietary intake of glucose because they are not responsible for a reduction in body weight of those animals to which they are administered. They have no effect on glomerular filtration or reabsorption of glucose because the glycosuria of diabetic patients treated with salicylates diminishes to an extent parallel to that of the reduction in blood sugar (10). Any observed salicylate effect of expanding the plasma volume is quantitatively inadequate to account for the fall in blood sugar (27). The formation of salicylate glucuronide is unlikely to be a mechanism of salicylate hypoglycemia (28). The conversion of glucose to liver and muscle glycogen might represent a possible mechanism of blood glucose lowering. But salicylates either have no glycogenetic effect or they deplete liver glycogen. Smith, Meade, and Bornstein (3) observed that large doses of salicylates reduced the glycosuria and blood glucose in the diabetic rat but caused no changes in the liver glycogen content and that in the normal rat the same treatment caused no alteration in blood glucose but a depression of liver glycogen. The glycogenolytic effect of salicylates in normal man and animals is responsible for the hyperglycemia of salicylate in-

TABLE I—EFFECT OF COMPOUNDS TESTED ON BLOOD GLUCOSE LEVELS OF DIABETIC RATS

Compd.	% Change in Plasma Glucose at 5 hr.[a]
Hypoglycemic Effect	
Salicylic acid, 4 mmoles/kg., s.c., oral	57.8
Benzoic acid, 4 mmoles/kg., s.c.	93.5
Anthranilic acid, 4 mmoles/kg., s.c.	90.8
Nicotinic acid, 2 mmoles/kg., s.c.	41.3
2-Hydroxynicotinic acid, 4 mmoles/kg., s.c.	47.5
2-Hydroxypyrazine-3-carboxylic acid, 2 mmoles/kg.	88.0
Indole-3-acetic acid, 4 mmoles/kg., s.c.	81.1
2-Thiophenecarboxylic acid, 2 mmoles/kg., s.c.	85.0
Hyperglycemic Effect	
Salicylamide, 4 mmoles/kg., s.c.	141.6
o-Mercaptobenzoic acid, 1 mmole/kg., s.c.	138.2
o-Nitrobenzoic acid, 4 mmoles/kg., s.c.	128.7
α-Resorcylic acid, 4 mmoles/kg., s.c.	150.4
m-Cresotic acid, 4 mmoles/kg., s.c.	143.2
o-Thymotic acid, 1 mmole/kg., s.c.	130.7[b]
3,5-Diisopropylsalicylic acid, 0.5 mmoles/kg., s.c.	138.3
o-Fluorobenzoic Acid, 1 mmole/kg., s.c.	163.3
1-Hydroxy-2-naphthoic acid, 1 mmole/kg., s.c.	227.9
3-Hydroxyisonicotinic acid, 1 mmole/kg., s.c.	129.4
No Significant Effect	
Nicotinamide, 4 mmoles/kg., s.c.	85.5
Salicylaldehyde semicarbazone, 4 mmoles/kg., oral	106.3
Salicylhydrazide, 2 mmoles/kg., oral	111.7
3,6-Dimethylsalicylic acid, 2 mmoles/kg., s.c.	105.9
3-*tert*-Butyl-6-methylsalicylic acid, 0.5 mmole/kg., s.c.	99.2
Phenol, 4 mmoles/kg., s.c.	93.5
Catechol, 1 mmole/kg., s.c.	105.9
Saligenin, 2 mmoles/kg., s.c.	83.9
2,4-Dihydroxybenzoic acid, 4 mmoles/kg., s.c.	90.7
Gentisic acid, 4 mmoles/kg., s.c.	80.1
2-Hydroxy-3-naphthoic acid 1 mmole/kg., s.c.	135.9
2-Aminopyrimidine-3-naphthoic acid 1 mmole/kg., s.c.	97.4
4-Aminopyrimidine-5-carboxylic acid 2 mmoles/kg., s.c.	121.8
4-Hydroxy-2-methylquinoline-3-carboxylic acid, 4 mmoles/kg., s.c.	117.1
Methyl-3-hydroxythiophene-2-carboxylate, 4 mmoles/kg., oral	116.1
Ethyl-4-hydroxy-2-methyl-thiazole-5-carboxylate, 4 mmoles/kg., oral	107.5

[a] *t* value significance never as much as 0.05. [b] % change in plasma glucose at 3 hr.

toxication. Niederlund (29) showed that both single and continuous doses of salicylates in the range of 230 to 810 mg./kg. body weight caused depletion of glycogen in the skeletal muscles and the myocardium of rabbits. Stowers (27) concluded that a long-term hypoglycemic action such as that induced by salicylates in man could not be explained by a change in distribution of or conversion to carbohydrates which could have only a transient action on blood glucose levels. Absence of relationship between salicylate uncoupling oxidative phosphorylation effect and hypoglycemia was reported by Gilgore (11) and Stowers (27). The hypoglycemic effect of salicylates does not appear to depend on either an insulin-like action or on liberation of insulin (10, 30–33). That the inhibition of lactate dehydrogenase by salicylates as a result of the reversible competition between them and pyridine nucleotide enzymes (NAD or NADP), is responsible for the hypoglycemia effect of the salicylate is unlikely because it is improbable that there is any difference in this inhibition in normal and diabetic animals. Attempts to correlate the metabolic effects of salicylates and their effects to diminish the protein binding of insulin and thyroxine (31, 34, 35) have not been convincing simply because the mode of action of these hormones does not fit the pattern of the hypoglycemic response to the salicylates.

Accumulating evidence suggests a reciprocal correlation between glucose utilization by peripheral tissues and release of nonesterified fatty acid (NEFA) from adipose tissue into the blood stream (36, 37). Carlson and Ostman (38) discovered that calcium acetylsalicylate in normal and diabetic subjects caused blood glucose and NEFA concentrations to fall.

Salicylate appears to depress NEFA release from adipose tissue. Inadequate glucose utilization by adipose tissue will induce a high level of NEFA in blood plasma (39). Observations by Shipp *et al.* (40) and Randle *et al.* (41) have shown that plasma fatty acids at high concentrations can inhibit the tissue utilization of glucose. These observations have been interpreted to suggest that the increase in NEFA occurring in diabetes can block the utilization of glucose from the blood stream. Thus, agents which will depress NEFA such as salicylate, should secondarily increase glucose utilization in diabetics and may lower blood sugar.

Table II illustrates the relationship between blood glucose and plasma NEFA. The effect of salicylic acid on plasma NEFA is seen within the first hour following administration, while the effect on blood glucose shows up toward the end of the third hour.

To determine whether or not the blood glucose

TABLE II—RELATIONSHIP BETWEEN BLOOD GLUCOSE AND PLASMA NEFA

D.R.[a]	Treatment	Blood Glucose Contents, mg./100 ml. 0 hr. Range	0 hr. Av.	Changes in % 1 hr.	3 hr.	5 hr.	Plasma NEFA Contents in meq./l. 0 hr. Range	0 hr. Av.	Changes in % 1 hr.	3 hr.	5 hr.
16	Saline control, 5 ml./kg., s.c.	86.9–350.1	188.2	117.0	115.6	115.8	0.55–1.65	0.92	93.4	104.3	129.6
10	Salicylic acid, 4 mmoles/kg., s.c.	94.2–350.7	182.4	103.7	91.6	72.7	0.51–1.27	0.81	17.9	53.1	93.0
8	Salicylamide, 4 mmoles/kg., s.c.	132.5–535.7	328.1	107.0	104.3	108.3	0.75–1.69	1.26	87.7	111.7	106.1
8	Benzoic acid, 4 mmoles/kg., s.c.	96.7–371.4	229.6	88.8	88.8	112.2	0.54–1.51	0.93	20.1	76.9	141.1
8	Thiosalicylic acid, 1 mmole/kg., s.c.	158.0–392.9	280.9	108.3	107.0	110.4	1.12–2.00	1.37	58.5	76.8	94.1

[a] Number of diabetic rats.

TABLE III—EFFECT OF EXOGENOUS NEFA TO BLOCK THE HYPOGLYCEMIC ACTIVITY OF SALICYLIC ACID IN DIABETIC RATS

D.R. Group	No.	Injected Serum[a]	Plasma NEFA Contents, meq./l. 0 hr. Range	0 hr. Av.	Changes in % 1 hr.	3 hr.	5 hr.	Blood of Injected Serum[a]	Blood Glucose Contents, mg./100 ml. 0 hr. Range	0 hr. Av.	Changes in % 1 hr.	3 hr.	5 hr.	t
I[b]	5	—	0.76–2.48	1.44	96.6	100.7	109.8	—	337.7–387.1	364.5	104.4	96.5	94.2	—
II[c]	5	—	0.70–1.69	1.30	24.1	39.8	53.0	—	198.0–408.6	349.5	96.3	69.2	45.4	0.005
III[d]	5	1.24–1.56	1.00–1.29	1.15	156.4	121.0	105.5	56.1–68.3	193.6–466.5	330.1	116.4	130.4	130.7	0.005
IV[e]	3	1.28–1.56	1.08–1.86	1.55	51.3	76.8	87.0	56.1–58.7	133.3–505.7	349.6	116.1	103.3	101.7	0.1

[a] The plasma NEFA and blood glucose contents of the blood samples, from which the serum was obtained and used for injection. [b] Normal saline control, 5 ml./kg., s.c. [c] Salicylic acid, 4 mmoles in 5 ml./kg., s.c. [d] Normal saline, 5 ml./kg. s.c and then serum from starved normal rats, 5 ml./kg., i.v. [e] Salicylic acid, 4 mmoles in 5 ml./kg., s.c. and then serum from starve normal rats, 5 ml./kg., i.v.

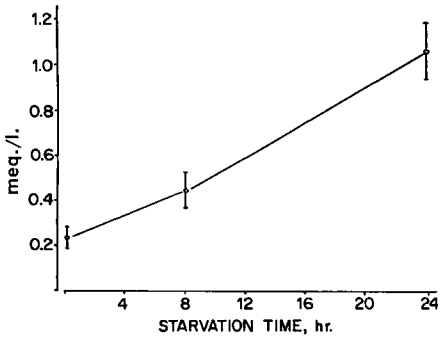

Fig. 2—Relationship between plasma NEFA level and starvation time (mean ± SD, 10 normal rats).

lowering capacity of salicylic acid is dependent upon the supression of plasma NEFA and therefore secondary to it, exogenous NEFA was supplied to salicylic acid pretreated animals.

The relationship between plasma NEFA level and starvation time is shown in Fig. 2. The effect of exogenous NEFA to block the hypoglycemic activity of salicylic acid in diabetic rats is seen in Table III. Thus, when the primary effect of salicylic acid to depress blood NEFA concentration is nullified by the addition of exogenous NEFA, the secondary hypoglycemic effect of salicylic acid is abolished.

SUMMARY

1. The carboxyl group of salicylic acid appears to be responsible for the hypoglycemic activity of this compound. The hydroxyl group at the *ortho* position is not imperative but beneficial to the hypoglycemic activity.

2. It would appear that the hypoglycemic action of salicylates is achieved through a primary effect to suppress the release of fatty acids from adipose tissues.

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Keyphrases

Salicylates—hypoglycemic activity
Structure-activity relationship—salicylates
Hypoglycemic acitivity mechanism—salicylates
Fatty acid, nonesterified—salicylate effect
Colorimetric analysis—spectrophotometer

Identification of a Prednisolone Derivative Interacting with Calf Thymus Histones

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Prednisolone dissolved in 0.2 *M* phosphate buffer, pH 7.4, was analyzed by TLC and found to undergo degradative changes. One of the products on the chromatogram was identified as 21-dehydroprednisolone which interacted with calf thymus histones. Minimal or no binding occurred with prednisolone and the carboxylic derivative. The transformation of prednisolone to 21-dehydroprednisolone was blocked by EDTA and facilitated by cupric ions. However, the binding of histones with prednisolone in phosphate buffer was depressed when cupric ions were added to the media. The results of this study suggest that prednisolone in phosphate buffer is transformed to 21-dehydroprednisolone which interacts with calf thymus histones.

THE BINDING OF glucocorticoids with calf thymus histones was shown to be dependent on a preliminary incubation of the steroids in phosphate buffer (1). In this study, the transformation of prednisolone[1] in phosphate buffer was studied. A derivative was isolated and identified as 21-dehydroprednisolone which interacted with histones. Comparative studies of the binding capacity of histones with 21-dehydroprednisolone and prednisolone in phosphate buffer at various pH values were carried out.

MATERIALS AND METHODS

The material and methods for the determination of the binding of histones with steroids were the same as previously reported. (1–3). 1,2,4-prednisolone-^{3}H with specific activity of 721 mc./mmole was purchased from Schwarz Bio-Research Inc., New York, N. Y. The steroids were purified on silica gel chromatogram (6061, Eastman Kodak Co., Rochester, N. Y.) before use. The 21-dehydro and 21-carboxylic derivatives of prednisolone were prepared according to the method of Lewbart and Mattox (4). The amount of steroid was estimated by measuring absorbance at 240 mμ or by the radioactivities and by the blue tetrazolium (5) and Porter-Silber reactions (6). A liter of phosphate buffer was washed with 100 ml. of 0.001% dithizone in CCl_4, v/v. The aldehyde group was detected by the Sawicki reaction (7).

Chromatogram sheets were developed by the ascending technique. Two solvent systems were used; namely, chloroform–ethanol, 96:4, v/v, and ethyl acetate–*tert*-butanol–5*N* NH_4OH, 50:40:20 by vol. The sheets were developed and dried at room temperature. The unlabeled steroids were detected by UV lamp. The labeled steroids were determined by cutting the sheets in 1-cm. sections and placing each strip in a counting vial. Ten milliliters of phosphor in toluene were added to each vial and the samples were counted as previously reported (1–3). Each steroid component separated on the chromatogram was extracted with 2 ml. of ethanol for 24 hr. at room temperature. The extract was dried under vacuum and the residue was dissolved in distilled water. The eluted components were rechromatographed. About 4% of the material remained at the origin and other metabolites were not detected. The binding of the steroid fraction with arginine-rich histones was performed as previously reported (1–3) with slight

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[1] Trivial names and abbreviations used: Prednisolone, pregna-1,4-diene-11β,17α,21-triol-3,20-dione; 21-dehydroprednisolone, pregna-1,4-dien-21-al-11β,17α-diol-3,20-dione; adrenosterone, androst-4-ene-3,11,17-trione; EDTA, ethylenediamine tetraacetate; dithizone, diphenylthiocarbazone.