that aminonucleoside nephrosis in rats is accompanied by increased rate of production of aldosterone. Further studies of this experimental syndrome have indicated that 4-androsten-3-one 17-spirolactone, an inhibitor of mineralocorticoids at kidney tubule level, promotes loss of ascites in aminonucleoside nephrotic rats without affecting their high rate of secretion of aldosterone (unpublished). By demonstrating that on maintenance doses of aldosterone, adrenalectomized nephrotic rats do not accumulate ascites whereas on higher doses of either aldosterone or desoxycorticosterone a state of fluid retention comparable to that observed in intact nephrotic rats is induced, the present data strongly suggest that hyperaldosteronism of aminonucleoside nephrosis is responsible for the presence of severe fluid retention. The same dosage of mineralocorticoids is without apparent noxious effects in adrenalectomized control rats.

Surtshin(6) reported severe fluid retention in adrenalectomized aminonucleoside nephrotic rats maintained on 1% NaCl in drinking water, but without any supportive therapy with adrenal hormones. We have confirmed this observation; this, together with results described above, suggests that fluid retention of aminonucleoside nephrosis is secondary to a decreased ability to excrete sodium. Hyperaldosteronism is one factor contributing to this disability.

Summary. Gross fluid retention and hyperaldosteronism are 2 characteristic features of aminonucleoside nephrosis in rats. Adrenalectomized aminonucleoside nephrotic rats receiving maintenance doses of aldosterone and corticosterone do not present fluid retention, whereas they do upon administration of aldosterone at doses equivalent to amount of this hormone secreted by adrenals of intact nephrotic animals.

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Received January 5, 1959. P.S.E.B.M., 1959, v100.

Cardiovascular and Respiratory Effects of Lethal Doses of Histamine and Compound 48/80 in Rabbits.* (24714)

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Compound 48/80 has been reported a potent liberator of endogenous histamine, capable of producing the main features of histamine and anaphylactic shock in several species(1,2). It is probable that 48/80 exerts pharmacologic and toxic effects which are unrelated to histamine released from tissues. Experiments have been made, using artificial respiration, to distinguish the effects of lethal doses of 48/80 and histamine on respiratory and cardiovascular systems of the rabbit, in which species 48/80 does not induce marked hypotension(2).

Methods. Albino rabbits of both sexes weighing from 3.0 to 4.6 kg were anesthetized with urethane (25% aqueous solution intravenously; 1.2 g/kg). The trachea was intubated with T-shaped glass cannula and respiration recorded semi-quantitatively on kymograph by connecting one of the outlets of the cannula to a recording bromoform manometer or to a rubber tambour. Femoral or carotid mean arterial pressure was recorded

* Acknowledgement is made to Parke, Davis Co. for financial support and for supplies of compound 48/80.

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kymographically with a mercury manometer. Artificial respiration using room air was administered by means of a standard respirator at 24 strokes/minute. Volume input was regulated to produce moderate chest expansion. All injections were made into marginal ear vein during 10 seconds. The volumes injected ranged from 0.4 to 1.5 ml. *Compound 48/80, Group 1.* Twenty-six animals received 2.25 mg/kg representing 1½ times the LD_{50} (1.5 mg/kg) predetermined in the unanesthetized animal. Artificial respiration was instituted one minute after respiratory arrest, or when blood pressure fell below 70 mm Hg even though feeble respiration was still present. At 10 minute intervals for approximately an hour, artificial respiration was interrupted for one to 2 minutes to determine whether animals would respire spontaneously. The procedure was then supplemented in some animals with use of certain analeptic drugs each administered to 3 different animals in the following manner: pentylenetetrazol 10 to 20 mg/kg, amphetamine sulfate 3.0 mg/kg and nikethamide 50 mg/kg injected intravenously in a volume of 2.0 ml over a 2 minute period. Upon completion of infusion, artificial respiration was again stopped for brief interval. In the absence of any breathing, artificial respiration was resumed. When possible a second injection was made 20-30 minutes after the first. *Group 2.* Responses to 48/80 in animals of Group 1 did not suggest that histamine liberation from tissues played an important causative role (see Results). Therefore 9 additional rabbits were injected with 3 times the LD_{50} of 48/80 (4.5 mg/kg) to determine whether responses similar to those induced with histamine could be elicited. In these animals, artificial respiration was started 30 seconds after injection of 48/80 and interrupted only at end of hour and every 30 minutes thereafter. Electrocardiograms were taken 2 minutes before injection and at 1, 5, 10 and 25 minutes afterward. *Histamine.* Ten animals received a lethal dose(3) of histamine phosphate (1 mg/kg) and were given immediate artificial respiration as in group 2 above. In 5 animals electrocardiograms were taken before and up to 10 minutes after histamine.

TABLE I. Effect of Artificial Respiration on Blood Pressure Changes Induced by a Lethal Dose of Compound 48/80* in Anesthetized Rabbits.

	Before art. resp.			After art. resp.	
Min. after inj.	0	4†	6†	8	30
Mean art. press., mm Hg	118‡	148	70	130	108

* 2.25 mg/kg i.v. (1½ times LD_{50}).
† Avg time of maximum hypertension and hypotension.
‡ Each value represents mean of 22-26 animals.

Results. Compound 48/80, Group 1. In all animals after 10-60 seconds, respiration gradually declined in rate and depth, occasionally after short interval of respiratory stimulation. In 19 animals, respiratory arrest occurred in average time of 5 minutes after injection, whereas in 7 animals respiration was only severely depressed. A rise in blood pressure started shortly after onset of diminishing respiration and attained an average maximum increase of 30 mm Hg in approximately 4 minutes (Table I). The rise was followed by precipitous fall. Upon starting artificial respiration, pressure reverted to control or above control values. Subsequently when artificial respiration was periodically halted, pressure again fell, recovering only upon resumption of artificial respiration. Two animals in this group receiving artificial respiration alone, reverted within an hour to spontaneous breathing adequate to sustain blood pressure within control limits. In only one experiment in which analeptic drugs were administered was there a return of spontaneous respiration; 2 injections of pentylenetetrazol had been given 20 min. apart after first hour. Actual effectiveness of pentylenetetrazol was questionable, however, in view of some results obtained using artificial respiration alone in this group and in Group 2. Other animals, whether receiving analeptics or not, usually died after approximately one hour following brief cessation of artificial respiration.

Compound 48/80, Group 2. When artificial respiration was started almost immediately, the initial pattern of blood pressure change seen in previous group no longer occurred (Table II). In 1½ to 3 hours, 5 animals resumed spontaneous breathing sufficient to maintain blood pressure above 90 mm Hg.

These animals had not been subjected to frequent periods of anoxia as in Group 1, and were maintained for longer periods and in some instances to the point where effects of drug had begun to diminish. Electrocardiographic records revealed no significant changes in cardiac rate or rhythm during first 25 minutes after injection.

Histamine. In all animals, blood pressure began to fall precipitously a few seconds after injection, reaching zero within 3-5 minutes. Even though artificial respiration was started 30 seconds after injection of histamine and continued for 10-15 minutes, blood pressure remained at zero, no spontaneous respiration was observed at end of experimental period, and no animals survived. Electrocardiograms recorded from 5 animals, one minute after injection revealed a variety of striking changes such as marked bradycardia, prolonged P-R interval, and varying degrees of A-V dissociation with QRS complexes that were abnormal with respect to amplitude and width. One animal exhibited brief intermittent episodes of cardiac arrest. Numerous abnormalities either persisted at 5 minutes or occasionally changed from marked bradycardia and partial A-V block to ventricular tachycardia. The 10 minute records usually revealed very slow electrical activity consisting mainly of P waves and independent widened QRS complexes of greatly reduced amplitude.

Discussion. Histamine and compound 48/80 produce death in the rabbit by different mechanisms. Lethal doses of 48/80 produced a striking depressant effect on respiration, the nature of which has not been determined. All animals could be kept alive for protracted periods following doses of 48/80 several times the LD_{50} as long as artificial respiration was administered. Blood pressure changes appeared to be directly dependent on respiratory effects since artificial respiration reversed or eliminated these changes. Dews *et al.*(2) reported a rise in blood pressure of the rabbit following intravenous administration of 2 mg/kg of 48/80. It is possible that the rise was due to asphyxia.

Absence of any serious effect of large quantities of 48/80 on the cardiovascular system in artificially respired animal was further indicated by a normal ECG up to 25 minutes after injection. Failure of certain analeptic drugs to improve respiration may have been due to inadequate dosage or to the possibility that analeptics do not act upon sites affected by 48/80.

TABLE II. Mean Arterial Pressure (mm Hg) of Anesthetized Rabbits Artificially Respired Immediately after Lethal Doses of Compound 48/80 and Histamine.

	Min. after inj.			
	0	5	10	30
I Compound 48/80 (4.5 mg/kg i.v.)*	118	111	111	106
II Histamine phosphate (1.0 mg/kg i.v.)	99	0	0	--

* Dose equals 3 times LD_{50}.

Each value for 48/80 group represents mean of 8-9 animals; for histamine, mean of 10 animals.

Following a lethal dose of histamine, however, blood pressure fell rapidly to zero and death ensued despite immediate administration of artificial respiration. Death may have been caused by known constrictor action of histamine on pulmonary arterial system of the rabbit with subsequent cardiac failure(4). The marked ECG abnormalities could have been due to such an effect coupled with adverse direct action of histamine on the heart. This series of events, possibly in conjunction with peripheral vascular changes, may account for circulatory collapse and absence of any beneficial effect from artificial respiration.

Respiration in the rabbit is affected during histamine poisoning(5). The influence of histamine on respiration was difficult to assess but in view of the very rapid onset of blood pressure and cardiac changes and failure of artificial respiration as a resuscitative measure, the primary effect appears to be cardiovascular. Respiratory defects could occur concomitantly with, or as a result of, initial deleterious action on the cardiovascular system. In any case they are not the primary reversible consequence which seems to be the case with 48/80.

Schachter(6) reported that intravenous injection of smaller doses of 48/80 in rabbits caused no significant increase in plasma histamine but that the compound did release histamine from the perfused isolated ears of this

animal. In our experiments doses several times the lethal dose elicited no responses which suggested release of significant amounts of histamine.

Summary. 1) In anesthetized rabbits, lethal doses of compound 48/80 produced marked depression of respiration with ensuing blood pressure changes that could be altered by administering artificial respiration. Blood pressure and electrocardiographic records in animals artificially respired soon after receiving doses several times the lethal dose revealed no evidence of adverse effects on the vascular system or on cardiac rate and rhythm. 2) Under similar conditions, however, a lethal dose of histamine produced a prompt and profound fall in blood pressure, marked changes in cardiac rate and rhythm and death despite artificial respiration. The effect appears to be primarily on the cardiovascular system. Respiratory effects are probably secondary to this main action. 3) The lethal action of 48/80 in rabbits is apparently not mediated by release of endogenous histamine since large doses do not elicit histamine-like responses.

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Received January 5, 1959. P.S.E.B.M., 1959, v100.

Co^{60} Vit. B_{12} Binding Capacity of Normal Human Cerebrospinal Fluid.*† (24715)

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As extension of continuing investigations in this laboratory on Co^{60} Vit. B_{12} combining capacity of serum and salive(1,2,3), the following study was made on ability of normal human cerebrospinal fluid to bind radiovitamin. Utilizing microbiologic assay methods, Sobotka, Christoff and Baker recently reported Vit. B_{12} content of cerebrospinal fluid from normal subjects to range from 0-30 $\mu\mu$g/ml(4). These authors found elevation of Vit. B_{12} in the cerebrospinal fluid and serum of about one-third of patients with multiple sclerosis.

Material and methods. Cerebrospinal fluid was obtained from 22 normal subjects with no apparent neurologic disorder, who were subjected to spinal puncture for injecting an anesthetic agent. The Co^{60} Vit. B_{12} had a specific activity of 0.823 mc/mg. To successive 1 ml aliquots of cerebrospinal fluid in Visking bags were added 1, 2, 5, 5, 10, 25, 50 and 100 mμg of radiovitamin. After incubation at room temperature for 2 hours, the mixture was subjected to exhaustive dialysis in cold, running tap water for 48 hours. All specimens were set up in duplicate, one to serve as a standard. Standards and dialyzed samples were placed each in 4 ml vials, filled with concentrated sulfuric acid to dissolve the casing, and radioactivity measured in a well-type scintillation counter. "Bound" Vit. B_{12} was calculated as activity retained in the bag after dialysis. Similar studies were made in 5 instances in which 1 ml of normal saline was substituted for cerebrospinal fluid, Co^{60} Vit. B_{12} added, and dialysis performed as de-

* This study was supported in part by grant from Nat. Cancer Inst., N.I.H., U.S.P.H.S.

† Co^{60} vit. B_{12} was provided by Merck and Co., Rahway, N. J.