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Effect of Adenosine, Inosine Triphosphate, Uridine Triphosphate, and Adenosine Triphosphate Upon the Plasma Volume of Intact and Adrenalectomized Dogs* (32726)

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This study is concerned with the marked increase in the plasma volume (PV) of intact and adrenalectomized dogs induced by iv injections of 100 mg of adenosine triphosphate (ATP), compared with the noneffect of similar doses of adenosine, inosine triphosphate (ITP) and uridine triphosphate (UTP).

Methods. Long-term, adrenalectomized, male dogs were used. They had been operated approximately 3 years previously and maintained in normal health by adequate substitution therapy during those periods when the animals were not in actual use as test objects for studies of adrenal insufficiency, stress-induced circulatory failure, or effect of vasoactive drugs. The methods employed for measuring alterations in the blood constituents have been cited in earlier publications from this laboratory (1). The PV was determined according to recommendations of Gregersen and Rawson (2) and Chien and Gregersen (3) using the dye T 1824 and the dye-tinged plasma read at 620 mμ in 10-mm cuvettes on a Beckman DB Spectrophotometer. The test substances were administered iv in 4 ml of water and injected slowly over 7 min; no untoward reactions were observed. The dogs were fasted 18–24 hours before sampling but water was allowed *ad libitum* during this interval. They were trained to lie quietly during the various manipulations required for injecting and withdrawing blood samples. Fifty min elapsed between injection of the compounds tested and sampling via the jugular vein.

Results. Essential data detailing the results obtained from adenosine injections are recorded in Table I A and B. Adenosine had no PV-raising action on either the long-term adrenalectomized or intact animal, instead a decline in PV followed its administration to dogs lacking adrenals. Table I sections C–F record data showing that inosine triphosphate

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TABLE I. Noneffect of iv Injections of 100 mg of Adenosine, Inosine Triphosphate and Uridine Triphosphate upon the Plasma Volume of Intact and Adrenalectomized Dogs Compared with the Marked Volume-Raising Action of Similar Doses of Adenosine Triphosphate.

Time after injection	Body wt. (kg)	Blood urea N (mg/100 ml)	Blood sugar (mg/100 ml)	Hb (gm/100 ml)	HCT (%)	Plasma volume (ml/kg)	Plasma volume Change (%)
			A. Adrenalectomized dogs				
			25 mg prednisolone and 100 mg adenosine; av 3 animals				
Control	21.01	15.5	85.0	15.56	47.0	44.3	
50 min	20.90	15.7	87.5	14.76	42.7	41.6	— 6.0
			B. Intact dogs				
			100 mg adenosine; av 3 animals				
Control	18.41	14.5	76.5	14.00	40.4	50.1	
50 min	18.37	15.3	76.0	14.77	40.2	49.3	— 1.5
			C. Adrenalectomized dogs				
			25 mg prednisolone and 100 mg inosine triphosphate; av 3 animals				
Control	22.01	14.2	74.1	14.76	42.7	43.6	
50 min	21.90	17.3	77.3	15.63	44.2	41.4	— 5.0
			D. Intact dogs				
			100 mg inosine triphosphate; av 3 animals				
Control	22.44	14.9	77.0	16.10	45.6	47.0	
50 min	21.77	12.4	79.0	15.99	44.8	45.2	— 3.8
			E. Adrenalectomized dogs				
			25 mg prednisolone and 100 mg uridine triphosphate; av 3 animals				
Control	23.52	17.8	78.3	14.28	42.4	41.7	
50 min	23.38	15.6	87.8	15.20	40.5	38.3	— 8.1
			F. Intact dogs				
			100 mg uridine triphosphate; av 3 animals				
Control	27.95	14.2	80.0	14.55	40.3	51.6	
50 min	28.06	11.2	75.0	15.60	44.8	47.6	— 7.7
			G. Adrenalectomized dogs				
			25 mg prednisolone and 100 mg adenosine triphosphate; av 14 animals				
Control	22.04	17.0	74.8	14.00	37.6	46.0	
50 min	21.27	15.1	76.2	13.02	33.5	53.9	+17.1
			H. Intact dogs				
			100 mg adenosine triphosphate; av 9 animals				
Control	22.35	13.8	80.1	16.46	43.0	51.1	
50 min	22.51	12.7	78.8	14.67	40.4	61.5	+20.3

(ITP) and uridine triphosphate (UTP) were also devoid of effect on the PV of both types of test animal. The UTP reduced the plasma volume somewhat. The average rise in the PV of 20.3% above control values which occurs within 50 min following injection of 100 mg of ATP in the intact dog, stands in striking contrast to the completely negative effect of similar dosages of steroid and ITP and UTP (compare Table I H with B, D, and F). However, when ATP is administered to adrenalectomized dogs, the compound is without effect on the PV unless accompanied by a small (25 mg) ineffective dose per se of prednisolone in which case the PV is increased an average of 17.1% based on the experimental use of 14 dogs (Table I G). No such elevation of PV follows when prednisolone is added to the

TABLE II. *In Vitro* and *in Vivo* Actions.

Sodium (water) pump	Increase of PV in intact and adx. dogs
1. Energy source for functioning of the pump: ATP.	1. Possible energy source: ATP plus glucocorticoids.
2. Pump activity inhibited by Ca and g-strophanthin.	2. PV increase inhibited by Ca and g-strophanthin.
3. Inhibition reversed by Mg, K, and Na ions.	3. Inhibition reversed by Mg, K, and Na accompanied by marked increase in PV.
4. Adenosine, ITP, and UTP without effect on the pump.	4. Adenosine, ITP, and UTP without effect on the PV.
5. Adrenal steroids (especially mineralocorticoids) effective agents for active sodium transport.	5. Adrenal steroids (especially glucocorticoids) highly effective for increasing the PV, mineralocorticoids relatively ineffective.

ITP or UTP injectates of dogs with or without adrenals (Table I A–F).

Discussion. Of the compounds tested, only ATP possessed the requisite properties for increasing the plasma volume of the fasted, intact, and adrenalectomized dog. In order to accomplish this in dogs lacking adrenals it apparently required activation or potentiation by C_{11} oxysteroids since it had no effect on the PV when glucocorticoid-type steroids were absent. Thus, dogs without adrenals do not raise their PV when given either small doses (25 mg) of prednisolone or large (100 mg) injections of ATP but promptly do so when the two are administered together.

On the other hand, intact animals with functional adrenals rapidly respond by elevation of PV following injections of either ATP or small doses of the steroid. Animals without adrenal cortices apparently lack, or seem to be deficient in, some factor present in the intact dog which enables the animal to respond by marked dilation of the peripheral vasculature thereby freeing any sequestered blood from restrictive barriers thus affording rapid increase in the volume of circulating fluid. This missing factor may be adrenal glucocorticoid. It has long been known that ATP possesses pronounced general arterial vasodilating properties when injected iv or intraarterially and is especially active in this respect when given by the latter route (4–8). The vasodilating action of ATP is considered by some investigators to be exerted directly upon the arterial vessels since denervation failed to diminish their vasoreactivity (7). Frohlich (9) observed that local infusion of sodium salts of adenosine mono-, di-, and triphosphate into the dog's limb greatly decreased the resistance to blood flow in the small vessels, primarily as the result of arteriolar dilation.

The *in vitro* action of various substances upon the factors regulating functioning of the enzymatic cation transport or sodium pump mechanism, located in cellular membranes, which is concerned with active movement of K into and Na (and water) out of cells (10–16), is oddly similar to the action of some of these same agents when tested *in vivo* for their activating, inhibiting and reversal of inhibition effect on the PV-raising action of adrenal glucocorticoids and ATP (17). Comparisons are shown in Table II. It seems improbable that the parallel behavior of these factors is merely coincidental and without significance but in any case the problem seems worthy of further study.

Summary. Adenosine, adenosine triphosphate, inosine triphosphate and uridine triphosphate were administered iv in 100 mg doses to intact and adrenalectomized dogs to test the ability of these compounds to increase the plasma volume. Only adenosine triphosphate was found to be effective. It readily induced marked elevation (av 20.3% rise) of the plasma volume of dogs possessing adrenal glands but had no effect on the plasma volume of the dog lacking adrenal cortices unless injected simultaneously with small (25 mg) but ineffective doses per se of the glucocorticoid prednisolone. The combined injectate prompt-

ly raised the plasma volume an average of 17.1% above control values based upon experiments on 14 adrenalectomized dogs.

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Permeability of Normal and Cataractous Rabbit Lenses to Glutathione* (32727)

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The presence of high concentrations of reduced glutathione (GSH) in the mammalian lens has been demonstrated by several workers. Incorporation studies with glycine ^{14}C have suggested that GSH is synthesized in the lens and that its half-life is 2–8 days (1, 2). Recently Reddy *et al.* (3) have shown that the average turnover rate for glutamic acid and glycine in GSH is 1.8% per hour and have indicated that 21.6 μmoles of glutathione are formed per 100 gm of lens per hour, based on the incorporation studies. Although the lens actively synthesizes GSH, the further fate of the tripeptide has not been defined. However, in experimental cataract produced by cataractogenic agents and in senile cataract, the content of reduced glutathione is drastically diminished (1, 4, 5).

In the course of investigating possible mechanisms of senile cataract formation, a new *in vitro* method for producing cataracts has been developed. This consists of incubating lens in a culture medium (6) with added tyrosinase and tyrosine. The dopaquinone, formed as a result of the action of tyrosinase on tyrosine, probably enters the lens and oxidizes the reduced nicotinamide adenine dinucleotide phosphate (NADPH), the sulfydryl group of GSH, and proteins. The lens cultured in such a medium becomes opalescent and exhibits the same enzymic alterations and diminution in GSH content, which are found *in vivo* in the senile or experimental cataract.

Methods and Materials. Rabbits weighing 1.5–2.0 kg were sacrificed by injecting air into the ear vein. The whole eye was excised and the lens with intact capsule was removed by a posterior approach. The lenses were rinsed in the medium and blotted with moistened filter paper. One lens was placed into the culture tube containing 10 ml of culture medium (6), 300 enzyme units of mushroom tyrosinase (Sigma Chemical Company), and 1.0 mg of tyrosine; the other lens of the same rabbit was placed in the control tube which contained only culture medium. Prior to the addition of the lens, oxygen was bubbled into each tube for 5 min. The culture tubes were incubated for 22 hours under sterile conditions at 37°C.

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