of virus propagated in monkey kidney cultures. 3) Attempts have been unsuccessful to separate a hypothetical simian contaminant by adaptation of these agents to HeLa, KB and propagable amnion cells (to be reported later) and serial passage in these cultures. 4) Humans infected with these enteric agents (except for ECHO 3 and 10) have been studied serologically, and a substantial number have shown significant rises in HI antibody titers in the post-infection specimens.

Separation of the viruses into 2 groups according to the influence of 37°C incubation on the HA titer results in an interesting parallelism with the grouping of the ECHO viruses proposed by Hsiung and Melnick(6) on the basis of plaque morphology and the susceptibility of patas and rhesus kidney cells. The proposed ECHO virus group A contains all 3 of the ECHO viruses (3, 6, and 11) with HA titers depressed at 37°C while group B contains 2 (E7 and E12) of the 3 viruses with HA titers unaffected by the temperature of incubation. The third member of this latter group (E10) was not assigned to either group A or group B.

Summary. 1) Monkey kidney cell culture fluids containing ECHO viruses 3, 6, 7, 10, 11 and 12 and Coxsackie B_3 have been shown to be capable of agglutinating human Group O erythrocytes in relatively high dilutions. Evidence has been presented indicating that the hemagglutinating activity is specifically related to these agents, rather than to possible simian viral contaminants. 2) Preliminary findings suggest that the HA property resides in the viral particle, that it is absorbed by erythrocytes during the process of agglutination and may be eluted from them, exhausting red cell receptors during the process. Despite such similarities to the myxoviruses, additional data suggest that these agents do not belong to the myxovirus group. 3) Monkey kidney cell preparations of polioviruses 1, 2, and 3, ECHO viruses 1, 2, 5, 8, 9, 13 and 14 and Coxsackie A_9 failed to agglutinate human Group O cells, whereas preparations of ECHO 4 and Coxsackie B_1, B_2, B_4 and B_5 manifested HA activity in low dilution.

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Effects of a Quaternary Derivative of Atropine, N-Benzyl Atropinium Chloride. (23610)

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We have shown(1) that N-benzyl and N-phenacyl atropinium salts, as well as certain other compounds containing quaternary nitrogen atoms, are capable of overcoming the Sarin-induced decrease in twitch response of the indirectly stimulated gastrocnemius-soleus-tibialis anticus muscle group of the cat. The present work was undertaken to study some of the functional changes induced by these compounds.

Methods. Cats, rabbits and guinea pigs of unselected sex were used. Young, male mice weighing about 20 g were used in toxicity determinations. LD_{50} values were calculated by the method of Miller and Tainter(2). Cats and rabbits were anesthetized with sodium pentobarbital (30 mg/kg i.p. or i.v., respectively). A metal bellows manometer recorded blood pressure; intratracheal pressure was recorded from a side-arm cannula by a Marey tambour. ECG was recorded with a Sanborn Viso-Cardiette and EEG by a Grass Electro-

encephalograph. Gut activity was registered through a float actuated by a water-filled balloon in the duodenum. Atropinium salts were dissolved in physiological saline to make 1% solutions. Animals given repeated i.v. doses of N-benzyl atropinium chloride (NBA) received 0.5 to 10.0 mg/kg at 5 to 15-min intervals; before and after each dose they were tested for response to (a) central and peripheral vagal stimulations, (b) occlusion of carotid arteries, (c) i.v. injection of 3 μg/kg of acetylcholine bromide, and i.v. injection of 3 μg/kg of epinephrine hydrochloride. Anesthetized rabbits were used for kymographic recording of the contractions of the nictitating membrane, excited indirectly by square-wave stimuli (frequency 50/sec., pulse duration 0.1 msec., strength 10 v.) applied to the preganglionic fibers of the superior cervical ganglion. Isolated rabbit duodenum or frog rectus abdominis muscle was suspended in oxygenated Ringer-Locke solution or aerated Ringer solution, respectively. The strips were exposed to concentrations of NBA of from 0.1 to 100 mg/100 ml. Contractions were recorded kymographically. The response of the gastrocnemius-soleus-tibialis anticus muscle group of the cat was recorded as in our previous paper(1).

Results. NBA had an i.p. LD_{50} for the mouse of 62.5 ± 3.0 mg/kg and for the guinea pig of 105 ± 5 mg/kg. By rapid i.v. injection NBA had an LD_{50} for the mouse of 7.4 ± 0.2 mg/kg and N-phenacyl atropinium bromide (NPA) had one of 9.7 ± 1.5 mg/kg. For both compounds speed of injection by the i.v. route had a marked effect on toxicity. Thus, 15 mg/kg of NBA could be given i.v. to mice without fatality if spread over 5 min. For comparison, the i.p. LD_{50} of atropine sulfate for the mouse is 223 mg/kg and for the guinea pig is 200 mg/kg; the i.v. LD_{50} for the mouse is 75 mg/kg and is only slightly dependent on the rate of injection. Because the hypotensive effect of NPA also was greater than that of NBA and because we sought a therapeutic compound without too marked side effects, our study was confined then to NBA.

I.v. injection of 4 mg/kg of NBA into an anesthetized cat not only lowers blood pressure but also slows the rate of respiration as well as decreasing the tidal volume. To determine whether these effects are central or peripheral in origin, 2 cats were given intracarotid or intrajugular injections of 1 mg/kg of NBA. The results are summarized in Table I, which shows that intrajugular injection of NBA produced almost twice as much slowing of respiration and very nearly the same hypotension as intracarotid injection. It seems likely, therefore, that NBA exerts its effects on respiration and blood pressure through peripheral rather than central actions. This conclusion is supported for blood pressure by findings that NBA-induced hypotension cannot be prevented by prior atropinization, by bilateral vagotomy or by section of the cervical spinal cord with bilateral vagotomy. NBA (4 mg/kg i.v.) blocked responses of blood pressure and of duodenum to both central and peripheral vagal stimulations, to occlusion of the carotid arteries and to injected acetylcholine. It had no significant effect on responses to injection of epinephrine. Central vagal stimulation was still capable of slowing respiration although it had no effect on blood pressure. These findings suggest again that the principal actions of NBA are on autonomic effectors rather than upon their centers of innervation.

TABLE I. Effects of 1 mg/kg of N-Benzyl Atropinium Chloride, Injected into Carotid Artery or Jugular Vein, on Blood Pressure and Respiration in the Cat Anesthetized with Sodium Pentobarbital.

Drug effect	Intracarotid		Intrajugular	
	Cat 1	Cat 2	Cat 1	Cat 2
	%			
Slowed respiration	17	7	24	21
Depressed blood pressure	74	70	64	68

Duodenal activity was depressed by small doses (1 mg/kg) of NBA; larger doses (2 to 5 mg/kg) increased both intestinal tonus and motility. *In vitro,* NBA (0.1 to 100 mg/100 ml) increased the tonus of rabbit duodenal segments, this effect being blocked partially by atropine. NBA (0.2 mg/100 ml) blocked the stimulant action of acetylcholine (0.2 mg/100 ml) on isolated duodenum or relaxed the segment when applied after acetylcholine. In

both cats and rabbits, NBA (1 to 10 mg/kg) produced no striking alterations of the ECG or EEG patterns. Low doses produced an initial tachycardia, followed by definite slowing of the heart rate. Higher doses produced immediate slowing of the heart and a general lowering of the potential of the EEG record, with the appearance of occasional alpha spindles.

Although NBA appears to have no significant effect on central structures, a dose of 4 mg/kg lowers by 60% the isotonic contraction of the nictitating membrane in response to electric stimulation of the preganglionic fibers of the superior cervical ganglion. The response returns to normal within about 20 min after injection of NBA. Atropine sulfate (5 mg/kg i.v.) produced no more than 10% depression of the same response, lasting for less than 5 min. It is apparent, therefore, that NBA is much more potent in decreasing transmission of excitation from preganglionic fibers to the effector than is atropine itself.

Experiments with isolated frog rectus abdominis *in vitro* showed that atropine sulfate in a concentration of 5 mg/100 ml produced only a slight, temporary block of stimulation by acetylcholine although NBA in a concentration of 0.5 mg/100 ml blocked completely stimulation by acetylcholine. Similarly, in the anesthetized cat NBA (5 mg/kg i.v.) blocked the stimulatory effect of acetylcholine (50 μg/kg), injected intraarterially, on skeletal muscle. About 30 min. after injection of NBA are required for the response to acetylcholine to return to normal.

Discussion. It is apparent that NBA has some of the antimuscarinic properties of atropine: ability to block the effects of vagal stimulation on blood pressure and heart rate and ability to block the effects of acetylcholine on blood pressure, heart rate and duodenal tonus. Shea(3) has shown that NBA is about ¼ as active as atropine sulfate in inhibiting secretion by the rat's stomach. In addition, NBA has certain effects not elicited significantly by atropine: fairly marked lowering of blood pressure, block of transmission of excitation from preganglionic fiber to nictitating membrane, block of stimulation of muscle by acetylcholine and acceleration of recovery from Sarin-induced block of responsiveness of muscle to electrical stimulation of its motor nerve. NPA is similar to NBA, so far as we have studied it, but has a more pronounced hypotensive action and a greater over-all lethality.

The stimulation of the smooth muscle of the gut by NBA, because it occurs in isolated segments of gut, must be a direct effect of the chemical on the peripheral structure. The effects on heart rate, blood pressure and respiration also seem to depend in large part, at least, on peripheral actions. This is in accord with the observation that, in general, quaternary compounds do not enter the central nervous system readily.

If NBA has a modality of activity not possessed by atropine, it should be adjunctive to the latter drug in the prevention of mortality from administration of Sarin. In rabbits given both Sarin and atropine intravenously, 2 mg/kg of atropine sulfate protected 3 out of 6 animals given 30 μg/kg of Sarin; and 0 out of 6 animals given 60 μg/kg of Sarin; 5 mg/kg of atropine sulfate protected 3 out of 6 rabbits given 30 μg/kg of Sarin and 1 out of 6 given 60 μg/kg of Sarin. A mixture of 2 mg/kg of NBA and 2 mg/kg of atropine sulfate protected 4 out of 6 rabbits given 30 μg/kg of Sarin and 2 out of 6 given 60 μg/kg of Sarin. NBA appears, therefore, to be adjunctive to atropine, at least in the rabbit.

Summary. Quaternization of atropine by the benzyl or phenacyl group* increases the lethality of the compound rather markedly; the resultant compounds have the antimuscarinic properties of their parent but to a lesser degree. They possess, in addition, a hypopneic and a potent, but brief, hypotensive action. The latter is especially marked in N-phenacyl atropinium bromide; it is not mediated through the carotid sinus or carotid body mechanisms or the vagal centers, but is largely peripheral in origin. N-benzyl atropinium chloride (NBA) prevents stimulation of striated muscle by acetylcholine and prevents transmission of excitation to the nictitating membrane from preganglionic fibers of the superior cervical ganglion; NBA has,

* NBA and NPA were made by Mr. Lawrence J. Edberg of the Directorate of Medical Research.

therefore, antinicotinic properties which are lacking almost entirely in atropine.

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Amino Aciduria in Primates Following Irradiation.* (23611)

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The acute radiation syndrome presents a symptom complex that is dose dependent. In the lower lethal dose range for the primate, the metabolic damage is expressed in 2 phases, an initial phase of predominantly alimentary dysfunction and a later phase associated with the catabolism of body tissues. In both phases, nitrogen balance becomes negative, but little change in the total daily urinary nitrogen excretion takes place until as an antemortem event, the daily urine nitrogen excretion may be greater than during a pre-radiation control day.‡ Some qualitative change in the nitrogen partition occurs and amino acid excretion is increased. This increase has been stated to be due to the radiation injury sustained by the animal(1). This paper presents a study of the urine amino acid nitrogen excretion of 4 whole body irradiated monkeys and of the 2 survivors (after physiological recovery) when sham irradiated and given the same dietary intake as in their radiation experiment.

Material and method. Adult male imported Rhesus monkeys (*M. mulatta*), tuberculin tested and separately housed, were placed in a metabolic cage. Their body weights varied from 6.8-12.4 kg and they were in robust health. They were fed by hand on Parkes "41" rat cubes and hand watered, so an excellent estimation of food and water intakes was possible. After a control period, the animals were whole body irradiated or sham irradiated and the urine amino acid nitrogen (amino N) and total nitrogen (total N) excretion followed until death or obvious recovery. The urines, voided into flasks with toluene as a preservative, were collected at the same time each day and analyzed for amino N by the copper titration method(2), and for total N by a semi micro method(3). Several urines were subjected to paper chromatography(4). *Radiation factors.* One animal was exposed to X irradiation, 240 Kv, HVL. 1.2 mm Cu with stepped Cu/Al filter, F.S.D. 150 cm, dose-rate 9 r/min. and the other 3 to gamma radiation (Co^{60}, 1.3, 1.1. Mev.) dose-rates 13-15 and 103-105 r/min. With both the X and gamma irradiation, the radiation procedure was arranged so that the dose received was relatively homogenous throughout the animal (5). Measurement of dose-rate was made with a Victoreen 100 r dose meter at various selected positions that the animal would subsequently assume. Thiopentone anaesthesia was given for the X irradiation(6).

Results. The daily urine amino N excretion of the 4 irradiated animals is shown in Table I. Since amino N is but a part of the total urinary N excretion, total N excretion is recorded as well. The values for the 4 animals during the control period were in close agreement, with the exception of monkey C, a smaller animal.

Immediately following radiation, only monkey C showed an amino N excretion greater

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‡ Unpublished observations, Hunter, C. G.