Rabbit hepatocytes formed a significant percentage of rosettes with EAP (48.1 ± 2.1) while with E the cells formed 5 times less rosettes (9.9 ± 0.8). The majority of cells have multiple EAP over the surface or around the membrane (figures 2 A and C). Few cells showed cap-like location of EAP (figure 2B). The cells attaching less than 6 EAP or E were considered as negative (figure 2D).

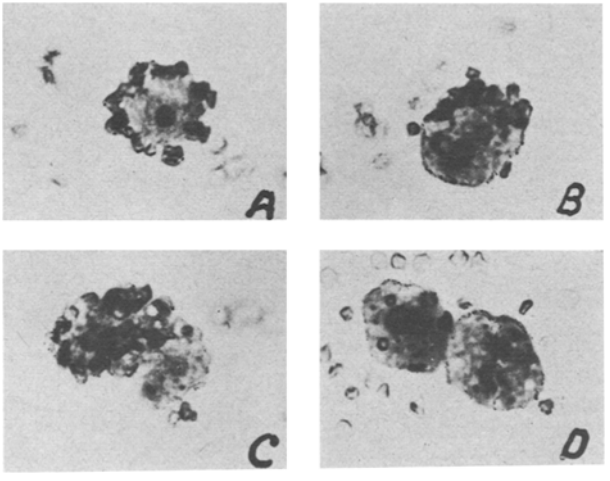

Fig. 2. Photomicrographs of rosettes formed by EAP (×400). A. Cell with ring-like distribution of EAP; B. cell with cap-like distribution of EAP; C. cell with multiple EAP over the cell surface; beneath this cell a negative hepatocyte can be seen; D. 2 negative cells. To 10^6 hepatocytes suspended in 0.1 ml of TC-199 medium containing 2% bovine serum albumin, 0.05 ml of EAP or E (10^9 cells ml^{-1}) was added in a small plastic tube. The mixture was centrifuged at 4 °C for 10 min at 250 g, and further incubated for 1 h at 37 °C. After adding 0.35 ml medium, the deposit was gently resuspended, fixed for 10 min with 0.2 ml 2% buffered formaldehyde and further treated with 0.1 ml 0.2% buffered toluidine blue. After 10 min more than 200 cells were counted.

The lower percent of hepatocytes binding EAP (50%) than that of cells stained with FITC-RSAP (90%) may be due to the different size of EAP (5 μm) and hepatocytes (30 μm), preventing some hepatocytes to form EAP rosettes during centrifugation. Rosette formation between hepatocytes and EAP was inhibited if the cells were previously treated with RSAP. The amount of RSAP which was able to inhibit rosette formation by 50% is lower than 10 μg 10^6 $cells^{-1}$. No significant inhibition of rosette formation was recorded with RSA (2 mg RSA 10^6 $cells^{-1}$).

The results presented here seem to show that on rabbit hepatocytes membrane there are receptors specific for the fixation and further manipulation of the polymerized albumin. The lack of the binding of RSA and RSA inability to inhibit the fixation of RSAP, as detected by fluorescence or by rosette formation, suggest that these specific receptors are able to discriminate between the different molecular forms of the albumin. The receptor for albumin on liver cell membrane functions as a binding site for in vivo polymerized albumin conferring to these cells the ability to remove the worn-out polymerized molecules from the circulation of the normal organism. In this way the hepatocytes would be able to select for catabolism the polymerized albumin from the native one. A similar mechanism of IgG catabolism by macrophages was suggested[9], and some evidence that IgG fixation and degradation proceed only after the aggregation of the molecules was presented[10]. The liver damage affects the ability of hepatocytes to remove the polymerized albumin from the circulation, therefore raising its level in serum. The polymerized albumin by its new antigenic sites is potentially able to induce the formation of specific autoantibodies.

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Radiation-released histamine in the rhesus monkey as modified by mast-cell depletion and antihistamine

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15 December 1976

Summary. 4000 rads of mixed gamma neutron radiation administered to rhesus monkeys released a significant amount of histamine into their circulation. When the monkeys were treated with a mast-cell histamine depleter (compound 48/80) for 4 days and then irradiated, no increase in circulating histamine was seen. When 48/80 was given 20 min after irradiation, only a slight increase in histamine was seen, indicating that 4000 rads had released most of the mast-cell histamine.

Increased blood histamine levels following ionizing radiation have been reported in rats and man[2-4]. These increased histamine levels appear to correlate well with hypotension[4] as well as with a reduction in the number of tissue mast cells[3]. Because ionizing radiation does cause disruption of mast cells, the suggestion has been made that mast-cell histamine release could be responsible for the observed hypotension[4].

These experiments have been designed to show a) the amount of histamine released by 4000 rads of ionizing radiation, b) the amount of histamine which is blocked from receptor sites by an antihistamine, c) whether the released histamine is of mast-cell or nonmast origin, and d) whether the histamine released could be responsible for the observed hypotension. These experiments were

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correlative to the cardiovascular and behavioral studies reported elsewhere[5,6].

Materials and methods. Healthy monkeys (Macaca mulatta) of both sexes, 2–3 years of age and weighing 3–4 kg, were used. Each monkey was anesthetized with sodium pentobarbital[7], and catheters were inserted into a femoral artery and vein. The monkey was allowed to recover and then was placed in a restraining chair. Few data were available on the histamine measurements of old-world monkeys because of the presence in these animals of high concentration of histaminase which rapidly catabolizes histamine[8]. Therefore, all histamine measurements were made only after the animals were treated with aminoguanidine[9], a histaminase inhibitor[10]. Discrimination between mast-cell and nonmast-cell histamine was accomplished using a specific mast-cell histamine releaser, compound 48/80[11,12], which has no direct effect on levels of nonmast-cell histamine. Treatment with compound 48/80 was continued until only minimal increases in blood histamine were noted.

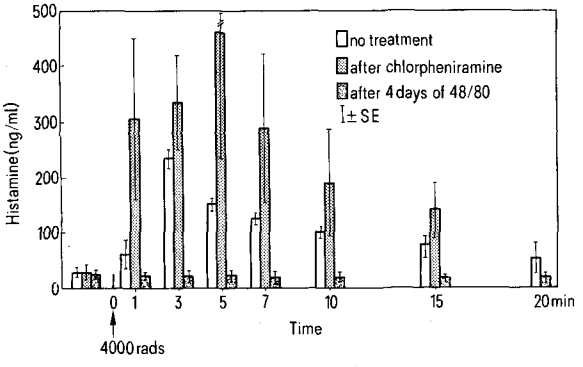

Fig. 1. Changes in blood histamine concentration following 4000 rads of ionizing radiation of 2 untreated monkeys, 4 monkeys given chlorpheniramine (3 mg/kg) 30 min before irradiation, and 4 monkeys treated with 48/80 (1 mg/kg per day) for 4 consecutive days.

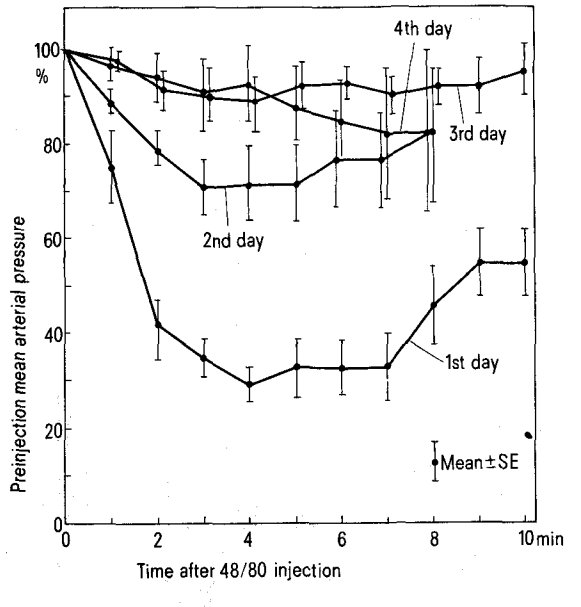

Fig. 2. Mean arterial pressure changes after injection of compound 48/80 (1 mg/kg) into 7 monkeys on 4 consecutive days.

Experiment 1. 2 monkeys were given aminoguanidine (10 mg/kg) i.v. to retard histamine catabolism, and 30 min later received a single, whole-body 4000-rad dose of mixed gamma-neutron radiation. Blood pressure was recorded continuously from a femoral artery. Arterial blood samples were taken at intervals for histamine determinations. Experiment 2. 4 monkeys were treated the same as those of Experiment 1, but in addition received an H_1 antagonist, chlorpheniramine[13] (3 mg/kg), 30 min before irradiation. Experiment 3. 7 monkeys were given animoguanidine and 30 min later, compound 48/80 (1 mg/kg) was injected i.v. Blood pressure was monitored before and for 10 min after injection of 48/80. Preinjection and 2-min postinjection blood samples were taken for histamine determinations. This experimental sesequence was repeated for 4 consecutive days. Experiment 4. 4 monkeys from Experiment 3 were irradiated with 4000 rads approximately 30 min after the fourth daily treatment with compound 48/80. Blood samples were taken for histamine determinations at selected times during this period. Experiment 5. 4 monkeys were given 4000 rads, and 20 min later aminoguanidine and 48/80 were administered. Blood samples were taken for histamine determination before and after drug injection. Blood sampling in the reactor exposure room was done by a remotely operated collecting device monitored via closed-circuit television. Each monkey received heparin (1000 USP units) to prevent blood clotting. The monkeys were irradiated in a pulsed, mixed neutron-gamma field (ratio of 1 neutron to 2.35 gamma) from the AFRRI-TRIGA Mark-F Reactor. Each animal was 1 m from the vertical centerline of the reactor core. The energy distribution of the neutrons as a percentage of fluence is as follows: 0.01–0.6 meV, 28.2%; 0.6–1.5 meV, 29.6%; 1.5–3 meV, 24.7%; and > 3 meV, 17.5%. A fast-scram

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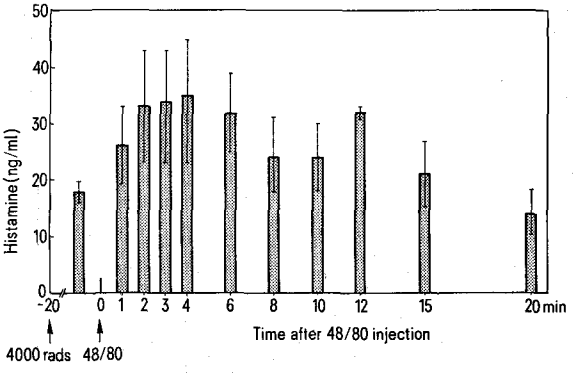

Fig. 3. Blood histamine concentration of 4 monkeys given 48/80 (1 mg/kg) 20 min after a 4000-rad dose of ionizing radiation.

technique was used for better reproducibility and to shape the pulse. Each monkey was given a unilateral, dorsal-ventral, whole-body irradiation delivered as a short-duration pulse (23 msec width at half-maximum height) of 4000 rads. Student's t test was used to determine the statistical significance of the difference between the means of the histamine values before and after drug treatment or irradiation. Probability values of less than 0.05 were considered significant. Histamine was assayed fluorometrically using the procedure of Shore et al.[8].

Results. The following results were noted. Experiment 1. Within minutes (figure 1) the concentration of histamine in the circulating blood began to increase and became maximal at 3 min. The concentration then began to decrease toward pre-irradiation levels. Experiment 2. Monkeys which are first treated with chlorpheniramine and then irradiation have a histamine concentration which is twice that of untreated animals (figure 1). Experiment 3. The result of partially depleting monkeys of mast-cell histamine can be seen in figure 2. The first dose of 48/80 produced a 298% increase in blood histamine levels. This was accompanied by a marked fall in blood pressure (to 30% of normal). The increase in blood histamine concentration and hypotension became less pronounced with each succeeding day of 48/80 treatment. On the fourth day histamine increased only 17% and the arterial blood pressure decreased only 20%. Experiment 4. Irradiation of monkeys which have been partially depleted of mast-cell histamine produced no significant increase in blood histamine levels (figure 1). Experiment 5. When 48/80 is given to untreated monkeys 20 min after irradiation, there is no significant increase in blood histamine concentration (figure 3).

Discussion. A 4000-rad dose of irradiation releases a significant amount of histamine. When an H_1 blocker, chlorpheniramine, is given before irradiation, the amount of histamine in the circulating blood is greatly increased. The difference in blood histamine concentration following irradiation with and without histamine blockers gives some indication of the amount of histamine that normally goes to receptor sites after release by this dose of radiation. We did not attempt to completely deplete the monkeys of mast-cell histamine with compound 48/80. After 4 consecutive days of treatment (figure 2 and table), the amount of mast-cell histamine released by the 4th dose of 48/80 was greatly reduced. This pseudotachyphylaxis is due to depletion of histamine stores rather than accommodation to 48/80. When the 4 repetitive doses of 48/80 were followed by radiation, there was no measurable increase in blood histamine levels as would be expected if the animals had been depleted of mast-cell histamine. When the animals were first irradiated and then given 48/80 (figure 3) 20 min later, the amount of histamine released was negligible; this is a further indication that 4000 rads of radiation had released most of the mast-cell histamine.

Conclusion. Based on circulating blood histamine levels, the following conclusions can be made: a) a 4000-rad dose of radiation releases a significant amount of histamine; b) the H_1 antagonist chlorpheniramine blocks the attachment to H_1 receptors of most of this histamine; c) the histamine released by 4000 rads of radiation is of mast-cell origin; d) a 4000-rad dose of radiation released most of the mast-cell histamine; and 48/80 given 20 min after irradiation produces only a light increase in circulating histamine levels.

Effects of electrical stimulation of periaqueductal gray matter on evoked potentials recorded in the primary somesthetic cortical areas of the rat

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Summary. The influence of focal electrical stimulation of PGM on primary cortical evoked potentials elicted by nociceptive peripheral stimulation was studied in anaesthetized and conscious rats. This analgesic electrical stimulation produces an abolition of cortical responses during its application and a significant decrease of the positive and negative amplitude waves after interruption of PGM electrical stimulation. Since these effects were observed in animals under barbiturate anesthesia and in conscious rats, they are interpreted as a supraspinal action on the primary pain pathway.

Deep analgesia, resulting from electrical stimulation of the periaqueductal gray matter (PGM) and other mesencephalic structures, was reported by Reynolds[2] and Mayer et al.[3,4]. Decrease or disappearance of the response to nociceptive stimuli was reported in these publications after stimulation of periaqueductal and periventricular gray matter. Similar results have been reported in the cat[5–7].

It has been postulated that these mechanisms may be similar to the central pharmacological effects of morphine[8], namely, the activation of a descendent inhibitory system which regulates the afferent impulses in the dorsal horn of the spinal cord. On the other hand, it has been shown that morphine has no effect on the amplitude or the latency in the primary pathway[9–12], whereas pentazocine modifies only latency[12].

Up to date the analgesic effect of focal electrical stimulation of the PGM has been studied by algesimetric tech-

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