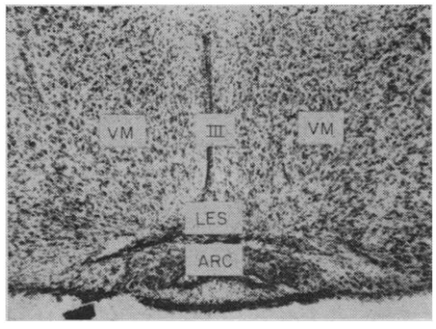

Fig. 2. Section of the brain of C3D2 female mouse, 3 months after intraperitoneal injection of 25 mg/kg of BPM. VM, ventromedial nucleus; III, third ventricle; LES, lesion; ARC, arcuate nucleus. [Courtesy Dr. R. A. Liebelt, Baylor University]

of lipids, there is initially a great acceleration in the oxidation of glucose, amounting to 100 percent at 1 week and 50 percent at 3 weeks after drug administration (Table 2). Despite the increased oxidation of glucose in vivo, there is also an increased fixation of glucose carbon into adipose tissues. Similar increases are also noted for ^{14}C-acetate 1 week after drug treatment, but there is subsequently a decline in the specific but not the total in vivo utilization of acetate. Preliminary investigations, with standard adipose tissue assay systems, indicate that there is no decline in the in vitro utilization of glucose or acetate for fatty acid synthesis during the period of rapid weight gain (0 to 5 weeks postdrug) but that subsequently these in vitro activities do decline significantly.

Since the obesity-inducing action of BPM seems to depend on the availability of the cyclic immonium derivative, it seemed reasonable to expect that the physiological effects result from alkylation reactions (*6*). To test this assumption we pretreated animals with *N*-acetylcysteine, a protective agent which we have demonstrated to effectively antagonize nitrogen mustard (*7*). Preprotection with *N*-acetylcysteine almost completely abolishes the obesifying effect of the BPM; only at high doses (40 to 50 mg/kg) is minor residual activity observed. As can be seen from the data (Fig. 1), weight gains on doses of BPM up to 30 mg/kg are restricted to the control level after *N*-acetylcysteine administration. In such experiments the LD_{50} of BPM is displaced from 30 to 35 mg/kg to ~45 mg/kg, a protection factor of 1.5, whereas the weight gain at 45 mg/kg is not significant. Thus, at equivalent biological doses as regards toxicity, the obesifying activity is virtually eliminated in the *N*-acetylcysteine treated animal, suggesting a specific antagonism between the -SH protective agent and the bipiperidyl mustard. Similar observations have also been made for the only other known chemical obesifying agent, gold thioglucose (*8*).

In summary, in numerous ways, the action of BPM seems to resemble that of gold thioglucose, which is known to produce hypothalamic lesions in the mouse (*9*). This resemblance has now been strengthened by the demonstration of hypothalamic lesions in BPM-treated mice. The lesions (Fig. 2) are located in the ventromedial and arcuate regions and appear very similar to those observed in gold thioglucose obese mice. Should the functional and anatomical resemblances be confirmed by further work now in progress, the chemical relations disclosed for both BPM and gold thioglucose suggest that -SH bearing sites in the hypothalamus may be targets for both drugs and may be involved in the appetite regulatory mechanism (*8*).

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1. The histologic examinations were performed on 60-g female obese C3H×DBA/2 mice by Dr. Philip Custer, pathologist, the Presbyterian Hospital, Philadelphia, Pa., to whom our appreciation is expressed.
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3. K. Prasada Rao and C. C. Price, unpublished observations submitted in partial fulfillment of requirements for the Ph.D. degree by one of the authors (K.P.R.).
4. We are deeply indebted to Dr. Charles C. Price for supplies of these drugs, as well as for the bipiperidyl mustard used in these experiments.
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10. We are deeply indebted to Dr. R. A. Liebelt (Baylor University) for the preparation and evaluation of the brain sections, a representative specimen of which is included in this report, and in particular, for the identification of the brain lesion.
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Increased Cardiovascular Reactivity to Angiotensin Caused by Renin

Abstract. *After a short period of tachyphylaxis, there is a marked and sustained enhancement of pressor responses to renin and angiotensin during chronic administration of renin.*

Subcutaneous injections of rat or hog renin to uninephrectomized rats elicit hypertension and vascular disease (*1*). Measurements of blood pressure obtained by sphygmography of the tail showed that the increase in pressure takes place only after a latent period of 2 to 3 days. This is similar to the result obtained by infusing subpressor doses of angiotensin; a period of normotension also precedes hypertension (*2, 3*). Since it is unlikely that hypertension is due to the accumulation of circulating angiotensin (*4*), the possibility that changes in cardiovascular reactivity could account for the hypertensive response was tested in conscious animals by directly recording the pressor responses to daily injections of renin.

Female Sprague-Dawley rats (150 to 200 g) maintained on commercial chow and tap water were used. After plastic tubing (PE 10) had been inserted into the lower aorta through the femoral artery, the animals were placed, for a 2-day recovery period, in harnesses which permitted free movements within the cage. They were then uninephrectomized to reduce renal antipressor activity. Mean arterial pressure was recorded during 8 hours daily with a Statham P23Db transducer connected to a Sanborn recorder. Crude hog or rat renin (40 Goldblatt units) was injected subcutaneously once every 24 hours. The results obtained by injecting these two preparations were similar and will be presented together. In some animals, test doses of angiotensin II (12.5 ng) and renin (0.05 units) in saline were injected intravenously through an intrajugular catheter. Daily base pressures were those recorded every morning, approximately 20 hours after a previous injection of renin. The experiments lasted up to 9 days. Data were obtained from 12 animals.

The first subcutaneous injection of renin elicited a slow and moderate increase in pressure followed by a plateau lasting for hours (Fig. 1). On the second, and sometimes the third day, the injection had little pressor ef-

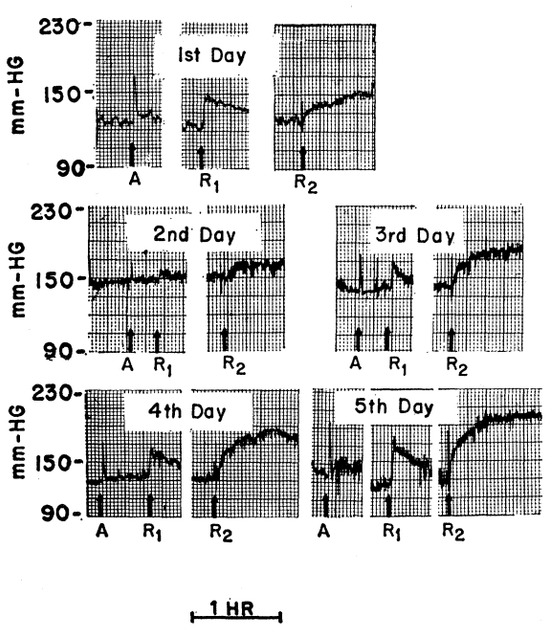
fect. However, starting usually with the fourth injection, responses occurred more rapidly and with greater intensity. By the fifth day the plateau at the height of the pressure increase was reached within 15 minutes instead of within one hour, and the response was about twice as great as that on the first day. Similar changes were observed after administration of intravenous test doses of renin. Responses were at first insignificant or markedly depressed, then they returned to normal and became enhanced. The pattern of responses to test doses of angiotensin was similar to, but not as evident as, that obtained with renin. Sharp, but transient, variations in arterial pressure resulting from handling of the animals at the time of injections sometimes obscured the acute pressor effect of angiotensin but did not interfere with the more gradual and sustained effect of renin. Base pressure prior to injection of renin was elevated on the second day but fell slightly below control level after the 3rd day of injections (Fig. 2). Examination of changes in pressor responses to renin and angiotensin and in base pressure over the period of 9 days showed an inverse relationship.

Whether the elevation in base pressure on the 2nd day accounted for the loss of response to renin was examined by testing rats at various intervals following the first injection. Equal doses of renin were given to different animals at the time of the plateau, during the return to the original levels, and at the 30th hour when the original levels had been reached. All pressor responses were markedly diminished; thus there appears to be no causal relationship between the level of the base pressure and the occurrence of tachyphylaxis to renin (*5*).

The significance of these experiments is not so much that they confirm the occurrence of renin tachyphylaxis following subcutaneous injection of renin, but that they demonstrate the subsequent development of an exaggerated responsiveness to renin and angiotensin. Tachyphylaxis is known to occur after repeated intravenous injections or infusion of renin (*5*). Since the prolonged pressor effect of subcutaneous administration resembles that caused by infusion, it is not surprising that tachyphylaxis also ensues. After single intravenous doses, tachyphylaxis lasts from 1 to 2 hours. During continuous infusion of large doses, arterial pressure first rises, then falls back to normal levels where it persists as long as the infusion is maintained (*6*). Since this period of normotension is due to tachyphylaxis, it is not clear why, under our experimental conditions, tachyphylaxis appears to subside gradually and then is followed by increased sensitivity.

Quantitative and qualitative differences in pressor responses suggest that during the period of exaggerated responsiveness another component is either superimposed on, or replaces, the direct pressor effect seen at the time of the first injection. There is increased evidence that angiotensin exerts two types of pressor effects, one direct, the other mediated by the nervous system (*7*). The first effect is responsible for, and is susceptible to, tachyphylaxis, while the other is immune to it (*8*). A nervously mediated pressor effect has been suggested to occur during infusion of subpressor doses of angiotensin (*3, 9*). It is, therefore, possible that what, under the present conditions, appears to be the remission of tachyphylaxis is the development, in spite of tachyphylaxis, of a nervously mediated pressor effect.

The increased cardiovascular reactivity to renin and angiotensin could also be related to changes in sodium metabolism. In man, increased sensitivity to the pressor effect of infused angiotensin has been attributed to sodium and water retention resulting from stimulation of aldosterone secretion (*10*). As in our experiments, increased sensitivity occurred around the 4th day of treatment. It is not clear, however, whether the same metabolic changes take place in the rat, a species in which angiotensin causes sodium loss instead of sodium retention (*11*) and in which it has a questionable effect on normal secretion of aldosterone (*12*). On the



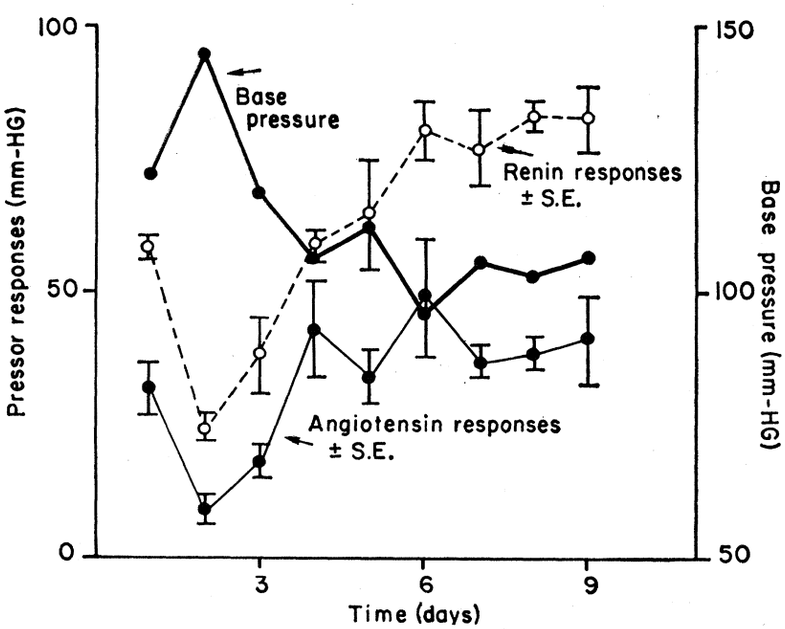


Fig. 1 (left). Pressor response in conscious rats receiving subcutaneous injections of 40 units of renin (R_2) to intravenous test doses of 12.5 ng of angiotensin II (A) and 0.05 unit of renin (R_1). Fig. 2 (right). Daily variations in pressor responses and in base (resting) pressure in response to intravenous test doses of angiotensin II and renin in rats treated with renin.

other hand, in the rat as in man, the pressor effects of renin or angiotensin are enhanced by sodium retention (*13*).

Although our experiments did not cause chronic hypertension in rats, they may explain how it develops when renin is administered every 8 instead of every 24 hours (*1*). It is likely that a combination of two factors contributes to hypertension: (i) a residual pressor effect at the time of each injection, and (ii) an increase in cardiovascular reactivity to renin which results in a gradual shift of base pressure to hypertensive levels. This view is in accord with the observation that hypertension can be elicited by chronic infusion of subpressor doses of angiotensin (*2, 3*).

Thus, from all available evidence it is clear that renin, like angiotensin, can elicit hypertension and vascular disease. The direct myotropic effect of angiotensin on blood vessels may play a relatively small role except in acute malignant hypertension. The existence of mediated pressor effects that persist in the presence of tachyphylaxis to angiotensin would give more significance to the early observation that renal hypertension is not remitted by experimentally induced renin tachyphylaxis (*14*).

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Immunization of Normal Mouse Spleen Cell Suspensions in vitro

Abstract. *Dissociated cells from the spleens of unimmunized mice were cultured with and without various mammalian erythrocytes. Spleen cell suspensions cultured with heterologous red cells developed levels of hemolytic plaque-forming cells only one $\log_2$ less than those seen in vivo. The reaction is specific for the in vitro immunizing erythrocytes. Antibody was demonstrated in the culture fluids.*

Development of techniques which permit immunocompetent cells to undergo normal immune reactions in vitro has been a major aim in the field of tissue culture. Several investigators have demonstrated such reactions occurring when tissue fragments (*1*) or dissociated cells (*2*) from immunized animals are cultured in the presence of antigen. It has also been reported that tissue fragments from unimmunized animals synthesize small quantities of antibody when cultured with extracts from macrophages exposed to antigen (*3*), when obtained from animals previously injected with either phytohemagglutinin or adjuvant and cultured with antigen (*4*), or when both spleen and thymus fragments are cultured together with antigen (*5*). Very recently it has been reported that tissue fragments from unimmunized animals can be stimulated to synthesize small quantities of antibody to bacteriophage (*6*). In all reports of the in vitro initiation of antibody synthesis in tissues obtained from unimmunized mice, the architecture of the tissue has been preserved. The data to be presented demonstrate that under simple culture conditions, dissociated spleen cells obtained from normal, unimmunized mice can be immunized in vitro and that a response occurs comparable in magnitude to that seen in vivo.

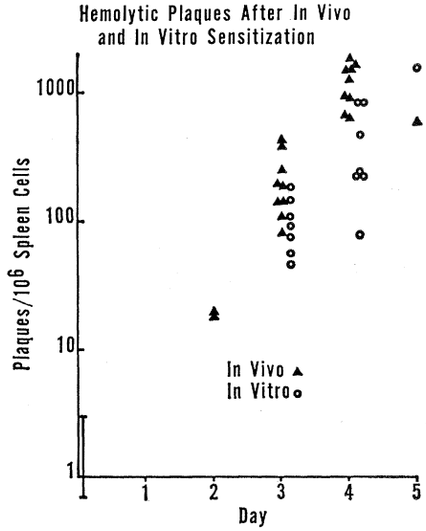

Fig. 1. Hemolytic plaques per 10^6 spleen cells against sheep erythrocytes. In vivo: mice injected intravenously with 0.2 ml 10-percent red cells. In vitro: 1-ml cultures contained 2×10^7 spleen cells and 1 to 2×10^5 sheep red cells. Background plaques (indicated by bar at day zero) = 0.3 to 3.0 per 10^6 spleen cells.

Cells were prepared by gently teasing apart the spleens of normal, unimmunized mice (*7*) in tissue culture medium. One-milliliter cultures containing 2×10^7 spleen cells in Eagle's suspension medium supplemented with "nonessential" amino acids, pyruvate, and 10 percent fetal bovine serum were placed in 35-mm plastic tissue-culture dishes (*8*) and incubated at 37°C in an atmosphere of 7 percent oxygen, 10 percent CO_2, and 83 percent nitrogen. Control cultures and experimental (antigen-containing) cultures were always set up from a single pool of dissociated cells; the experimental cultures contained, in addition to the spleen cells, 1 or 2×10^5 red cells of the appropriate type. The cultures were eccentrically rotated in the horizontal plane at 45 rev/min on a gyrorotary shaker. We added 150 μl of a nutritional cocktail (*9*) to each culture daily. Cultures were harvested and assayed at 3, 4, and sometimes 5 days. Cells were tested for antibody synthesis by the hemolytic plaque technique (*10*), and culture fluids were examined for released antibody. Suspensions of cultured cells were tested in duplicate at several cell concentrations in order to facilitate accurate counting. Forty to 50 percent of the cells planted were recovered; no viability tests were performed. The results are expressed as plaques per 10^6 total recovered cells.

The data presented in Table 1 show that large numbers of plaque-forming cells develop when normal mouse spleen cells are cultured with sheep erythrocytes. In seven consecutive experiments, using four different strains of inbred mice, increases of plaque-forming cells up to 1000 times the background were observed. A marked increase usually occurred between the 3rd and 4th days. It is of considerable interest that significant increases of plaque-forming cells were also observed in spleen cell suspensions cultured in