been kept under conditions of controlled illumination, the times of light and dark being such that ovulation took place between 11 A.M. and 3 P.M. (*3*). The average interval, in any one rat, between ovulation and the penetration of all the eggs was estimated in the same manner and again was found to be about 5½ hr.

Similar observations, as yet unpublished, on 150 mice kept under controlled lighting conditions showed that, for any one mouse, the average interval between ovulation and the penetration of the majority of the eggs was 6 to 7 hr.

In a fourth experiment, 14 oestrous rats that had been kept under normal colony conditions were not permitted to mate until 9 to 9:15 A.M.—that is, between 5 and 9 hr after ovulation. We have shown elsewhere (*3, 4*), that, under such circumstances, sperm penetration into the eggs does not begin until 2 to 4 hr after mating and then reaches completion, in any one rat, in 1 to 2 hr. The eggs of these rats must, therefore, have been penetrated by sperms between 8 and 15 hr after ovulation. The rats were allowed to carry their litters to term; a total of 91 apparently normal young (45 ♂♂ and 46 ♀♀) were born and later weaned, corresponding to a mean litter size of 6.5. The mean litter size at weaning of normally mated rats in this colony is 7.7.

Blandau and Jordan (*5*) obtained comparable results from rats artificially inseminated about 6 hr after ovulation; they recorded a mean litter size of 4.6 as compared with 6.7 when insemination was effected before ovulation. When allowance is made for the interval of 3 to 6 hr, which, as already mentioned, occurs in rats between late mating and the penetration of all the eggs, it is seen that the actual time of sperm penetration in their experiments must have been 9 to 12 hr after ovulation.

Mating between 8 and 10 A.M. on the morning of ovulation has been found to result in dispermy in about 9 percent of fertilized rat eggs, and this condition almost certainly leads to triploidy in the embryo (*6*). Chromosome counts on testis squash preparations from 40 to 45 male rats born in the fourth experiment just described revealed no instance of triploidy. This supports the conclusion of Beatty (*7*) that triploid embryos seldom survive to term and partly accounts for the decreased litter size observed in rats after delayed mating.

In short, we have found that, when mating was unrestricted, sperm penetration of all eggs in any one rat was not completed until nearly 6 hr after ovulation, and even when penetration was experimentally delayed until 8 to 15 hr after ovulation, the prenatal loss attributable to the delay was less than 16 percent. The fertile life of most of the eggs in our rats, therefore, was at least 10 hr. The same may have been true also for the mouse eggs, but this was not tested; however, it was shown that in mice allowed free mating, as under colony conditions, the mean interval between ovulation and the penetration of most eggs in any one mouse was as long as 6 to 7 hr. Now, Runner and Palm (*2*) found that when the results of transferring eggs 4 hr after ovulation were compared with those of transferring eggs at the time of ovulation, there was a loss of more than 80 percent of the young. This is in strong contrast to prenatal losses from all causes of 23 percent (unpublished data) and 30 percent (*8*) observed in apparently normal mouse colonies. The much larger loss observed by Runner and Palm must have been in some way associated with manipulation of the eggs *in vitro*, and we conclude that the resistance of eggs to the injurious effects of conditions *in vitro* must diminish rapidly with age.

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Nonidentity between Pepsitensin and Hypertensin Revealed by Paper Electrophoresis

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Croxatto and Croxatto (*1*) reported the production of a new vasoconstrictor and hypertensive substance when plasma globulins were incubated with pepsin at 38°C and *p*H 2 to 6. This substance, which was named pepsitensin, was subsequently studied by several authors and found to present chemical and pharmacological properties very similar to those of hypertensin (*2*). The only difference between the two substances was found by Braun-Menendez and coworkers (*3*), who showed that crude preparations of pepsitensin are not destroyed by hypertensinase from dog's red blood cells (RCH). However, this was shown to be due probably to the presence in crude pepsitensin of hypertensinase-inhibiting substances; in fact, Vasquez (*4*) succeeded in inactivating as much as 50 percent of a purified pepsitensin by incubating it with a dose of RCH sufficient to destroy completely an equipressor amount of hypertensin. Subsequently, we (*5*) were able to purify further pepsitensin and *completely* abolish its pharmacological activity by a dose of RCH that destroyed hypertensin as well. The similarity between both substances was further strengthened by our work when we found that a mixture of both could not be resolved in its components by paper chromatography with three different pairs of solvents (butanol : acetic acid : water, 4 : 1 : 5; acetone : acetic acid : water, 5 : 1 : 4; dioxane : butanol : water 10 : 10 : 5).

We now report the results of the comparative study

of pepsitensin and hypertensin by means of paper electrophoresis (*6*), using a slight modification of Durrum's technique (*7*). Three strips of No. 1 Whatman filter paper (2.5 by 40 cm) were suspended by their central parts on a horizontal supporting glass rod and their ends were dipped into positive and negative electrode vessels containing a glycine/HCl buffer solution of *p*H 2.5 and 0.1 ionic strength.

After the strips were soaked with the buffer solution, a potential difference of 3500 (*8*) was established between the two poles and the current was maintained for 30 to 60 min and then interrupted. Ten microliters of equivalent solutions (130 Indianapolis units/ml) of pepsitensin and hypertensin was then placed on the center position of strips 1 and 2, respectively, and a mixture containing 5 µlit of each solution was applied to strip 3. The whole system was placed in a chamber where air was completely replaced by nitrogen. The electric current was maintained for 5 to 6 hr, the voltage being kept constant through a voltage regulator; the current usually rose from 6 ma at the beginning of the experiment to 8 ma at the end. The paper strips were then dried at room temperature and cut into pieces 1 cm long that were assayed on the isolated guinea-pig ileum.

The response of this preparation has been shown to be a very sensitive method for the detection of hypertensin (*9*) and pepsitensin (*10*). As Fig. 1 shows, both substances migrated toward the cathode at *p*H 2.5; but pepsitensin had a greater mobility than hypertensin, and they were separated when a mixture of the two was submitted to electrophoresis. Thus, hypertensin and pepsitensin seem to be such similar peptides that their mixture cannot be resolved by paper chromatography with three pairs of solvents, but it can be resolved through paper electrophoresis.

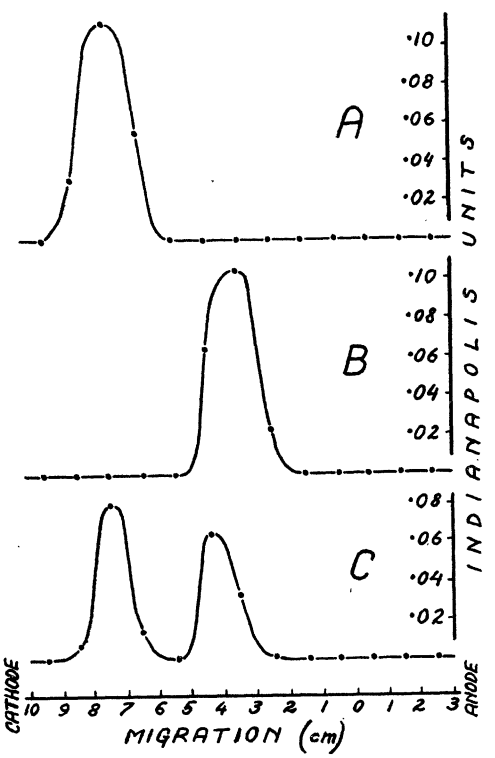

Fig. 1. Migration of pepsitensin, hypertensin, and a mixture of both on paper electrophoresis: *A*, pepsitensin; *B*, hypertensin; *C*, pepsitensin plus hypertensin. Potential difference, 350 v; time, 6 hr; buffer glycine/HCl, *p*H 2.5, ionic strength 0.1. The substances were localized by testing segments of paper on the isolated guinea-pig ileum.

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A Method of Assessing Experimental Pulmonary Edema

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A technique for assessing pulmonary edema has been successfully used at Medical Laboratories in recent months. The original technique was first shown to me some years ago by Hermann Rahn (*1*) of the University of Rochester, whose interest in respiratory physiology has led him, among numerous other activities, to preserve the lungs of a great number of species of animals by drying them on a compressed-air line. Lung, when minus tissue water and blood and inflated with air, becomes parchment-like yet preserves its morphological features and remains intact for many months with no other treatment. After some years of using this technique to make preparations, it has been adapted to the study of the dynamics of edema formation.

In order to measure the rate of development and the amount of pulmonary edema produced by chemical or physical agents in experimental animals, one must know the weight of the nonedematous lungs. This value is usually calculated from average figures taken from a large sample of untreated animals. Since the weight of the lung as percentage of body weight is quite variable among individuals of all species, obtaining an average figure means the use of considerable numbers of control and experimental animals in order to obtain a reliable figure. We are describing