of hybrid vigor, or heterosis, until it is pointed out that the ovarian hormones are notably more potent than are the hormones of the testes with respect to radioprotection. It is even known that the male hormone is deleterious, with respect to radiation exposure (*2*), and combining the male hormone factors from two strains would tend, in all probability, to counteract any otherwise heterotic effect. In contrast with this, since the ovarian (female) hormones are beneficial (with respect to radiation resistance) one would logically expect that, added to other heterotic factors, the resulting hybrid females would be statistically more radioresistant than females of either parent stock. This is in fact our finding.

One conclusion is obvious—namely, that the combination of the haploid $C_{57}$ black genome with the $CF_1$ white genome shows recessivity of the higher sensitivity of the latter, so that the hybrids exhibit radiosurvival in percentage values approximately three times that of the pure whites. Since, also, the male hybrids showed better tolerance than did the pure blacks (even though the finding is not statistically significant), one must admit to the probable influence of heterosis.

A recent paper by Uphoff (*11*) shows that $F_1$ marrow from a hybrid cross had more radioprotective value than did the marrow from either of the parental strains. The heterotic effect, therefore, probably pervades the entire hybrid.

The hybrid offspring obtained when two strains of mice are crossed ($C_{57}$ and $CF_1$) have higher survival values than do either of the pure strains when exposed to whole-body $LD_{50/30}$ day x-rays. The increase is significant for the female offspring but not for the males. The results are explained in the case of the males on the basis of the combined and deleterious male tendencies being inadequate to counterbalance completely the beneficial effects of heterosis; the combined and beneficial female tendencies from the two strains, plus heterosis, resulted in statistically significant survival values for the $F_1$ females.

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3 October 1957

## Induction of Neural Tissue in Ventral Explants from Frog Gastrulae by Carbon Dioxide Shock

If the speculation that the differentiation of cells depends in part upon cellular protein synthesis has any validity, then the possibility exists that the culture of ventral mesoderm and ectoderm of the early *Rana pipiens* gastrula (stage 10) under conditions which might accelerate protein synthesis conceivably might also effect the type of cell differentiation. In the vast majority of control experiments, the ventral parts of the frog gastrula showed differentiations of mesenchyme, epidermal and blood cells, and also oral suckers, when cultured at 17°C in small stender dishes containing about 10 ml of a buffered saline solution (Niu-Twitty solution) (*1*) for periods of 6 to 14 days. Neural, muscular, nephric, or chordal tissues appear in less than 2 percent of the cultures. At the end of the culture period the explants were fixed in Bouin's fixative, sectioned at 10 μ, and stained with hematoxylin eosin.

In the various experimental series of cultures, the ventral explants were cultured at higher temperatures, in various nutrient media and were also subjected to brief shocks with alkaline and acid media. For the control and experimental series a total of 230 cultures, which included 1900 explanted tissues, were made. The aim of these experiments was to increase the level of metabolism, or of available nutrient, and thus possibly to accelerate the synthesis of cytoplasmic proteins and determine the effect upon differentiation (*2*).

Possibly the most direct means of raising the rate of metabolism of the ventral explants is to culture them at higher temperatures. Although Gilchrist (*3*), Huxley (*4*), and Margen and Schechtman (*5*) applied local high-temperature stimuli to ventral regions of whole eggs without altering morphogenesis, this method has the disadvantage that it may not overcome any dominance factors that might emanate from the differentiating dorsal structures. This would not be a problem in culturing the isolated ventral tissues at the higher temperatures. When gastrula ventral mesoderm-ectoderm was cultured at temperatures of 28, 30, and 33°C for 3 to 4 days and then at 17°C for 6 more days, differentiation was merely equivalent to that of the control cultures. Thus it appears that higher temperatures alone, although they certainly raise the level of respiration, cannot alter the prospective fate of these tissues. However, it is known from experiments in vitro (*6*) that higher temperatures alone cannot increase the extent of solubilization of yolk platelets.

Another possible means of attempting to accelerate protein synthesis in the explants is to supply the cells with nutrient in the culture medium and thus attempt to bypass the yolk as the nutrient source. To this end, bits of ventral mesoderm-ectoderm from early gastrulae were cultured in various concentrations of a nutrient medium containing all the essential amino acids, most of the water-soluble vitamins, glucose, and each of the nucleotides; in saline extracts from hatched larvae, adult frog hearts, brains, and muscle; and also in livetin (the water-soluble yolk protein) dissolved in a *p*H 5.7 acetate buffer-saline medium. None of these series of explants showed greater specialization than the controls cultured in Niu-Twitty solution. The combination of added nutrient and culture at higher temperatures also failed to induce the formation of dorsal tissues in a significant number of cases.

Acid and alkaline shocks have been utilized in eliciting the formation of neural tissue in isolated ventral tissues of urodele gastrulae (*7*), but such experiments have not yet been successfully carried out with the frog embryo. Holtfreter (*8*) has demonstrated that a *p*H below 4.0 and above 9.6 partially solubilizes yolk platelets, and I have put forth the idea that solubilization of protein, including ribonucleoprotein, from the yolk platelets and the initiation of embryonic differentiation may be causally related (*6*). In order to ascertain whether inductions could be obtained in the anurans with low and high *p*H, ventral explants were exposed to 0.02 percent $NH_4OH$ in tap water (*p*H 11.0) for 3 to 6 minutes, washed several times in sterile saline, and cultured for 6 to 12 days. The acid shocks were carried out by bubbling $CO_2$ into the nonbuffered "A" part of the culture medium until the *p*H was 3.7, and explants were left in this acidified medium for periods ranging from 10 minutes to 1 hour and then cultured in the normal saline medium for 12 days. Most of the $CO_2$ shocks were for 10 or 20 minutes since longer shocks sometimes killed the explants.

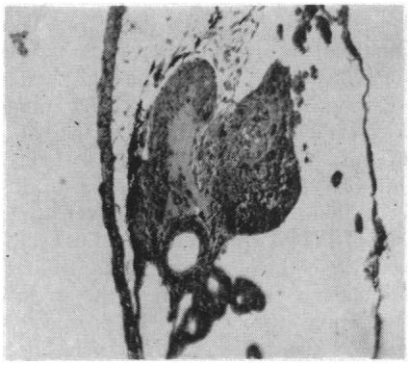

Fig. 1. Neural and nephric differentiation in a ventral explant that received a $CO_2$ shock.

The use of $CO_2$ is particularly advantageous because this gas can readily penetrate into the cells and because it does not disaggregate the cells or cause the marked cytolysis that followed the alkaline shock treatments. Modification of the "A" part of the culture medium with a 0.005*M* acetate buffer to a *p*H of 3.7 was not effective in inducing dorsal tissues.

No dorsal structures were observed in the explants that received alkaline shocks, but neuroid tissue and parts of the brain were frequently noted in explants that had received $CO_2$ shocks. An example of such differentiation is illustrated in Fig. 1. Less frequently, muscle, nephric tubules, and notochord were present in the explants. Of the ventral tissues that received $CO_2$ (*p*H 3.7) shocks, 50 percent showed neuroid tissue, 40 percent had well-defined brain vesicles, while in 10 percent of the cases no dorsal structures were present. The latter percentage is far above that of such tissues appearing in the other types of culture. One possible interpretation of the mechanism of $CO_2$ induction is that the acid shock released soluble protein and ribonucleoprotein from the yolk platelets (*6, 8*) and that this initiated the synthesis of cytoplasmic protein and subsequent differentiation of the dorsal tissues.

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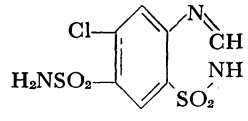
4 November 1957

## Electrolyte Excretion as Influenced by Chlorothiazide

In 1953 we reported that *p*-carboxybenzenesulfonamide produced (i) both natriuresis and chloruresis in dogs and (ii) that there was a causal relationship between the (plasma) concentration of drug presented to the kidney and the saluretic effect (*1*). These two attributes of that compound appeared to be inconsistent with the more classical concepts of the nephrotropic action of a carbonic anhydrase inhibitor (*2*). That agent was not sufficiently potent to warrant extended clinical study.

This premonitory indication that saluresis might be a clinically important attribute of a potent carbonic anhydrase inhibitor that fulfilled certain specific pharmacodynamic criteria has influenced our studies of renal electrolytes. In addition, the secondary attributes for an essentially specific nephrotropic agent of this category have been considered by us to include good absorption from the gastrointestinal tract, a volume of distribution essentially limited to extracellular fluid, a high concentration ratio of the compound in nephron/plasma, and high carbonic anhydrase inhibitor activity (*1*).

In the course of this study, it was found that an unusual heterocyclic acid increased substantially the excretion of sodium and chloride by the dog. This agent was synthesized by Novello and Sprague (*3*), who identified it as 6-chloro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide and assigned to it the following structure:




The compound is referred to herein as chlorothiazide or Diuril (*4*).

Qualitatively, at equal dosages and under similar experimental conditions, chlorothiazide induces changes in electrolyte excretion that more nearly resemble those known to be caused by the organomercurial diuretic agents than the response generally considered to attend the administration of a carbonic anhydrase inhibitor. Under reasonably normal conditions of acid-base balance, the effect of chlorothiazide is to increase the excretion of sodium and chloride preponderantly. The excretion of potassium and bicarbonate is increased slightly, if at all. However, the effect of the drug on the ratio of sodium to chloride excreted per unit time varies according to the state of electrolyte balance of the dog.

In the two experiments summarized in Table 1, the dogs were pretreated on three successive days with moderate amounts (3.0 g/day) of $NaHCO_3$ or $NH_4Cl$, the last dose being administered just prior to the control phase of the test. When the animal was pretreated with $NaHCO_3$, chlorothiazide caused a substantial increase in chloride excretion that was quite inadequate to cover the profound increase in sodium elimination. The increase in urinary *p*H reflects a substantial increase in bicarbonate excretion in the drug phase which more elaborate renal clearance experiments have demonstrated. In the $NH_4Cl$ experiment, chlorothiazide caused a marked increase in chloride excretion which was essentially equivalent to the amount of sodium eliminated. Under the conditions of this experiment, the excretion of bicarbonate is negligible and the effect on potassium elimination or urinary *p*H is not impressive.

The oral or parenteral administration of chlorothiazide to dogs made edematous by an excessive daily intake of a mineralocorticoid, NaCl, KCl, and water resulted in a prompt loss of fluid with reduction of weight to normal only as long as the drug was administered (Fig. 1). During the 100-day test, the apparently normal dog received 6 mg of 9α-fluorohydrocortisone, 2 g of NaCl per kilogram, and 150 mg of KCl plus 1000 ml water orally per day in addition to the Na and K contained in its diet. During the coadministration of 5 mg/kg of chlorothiazide orally twice daily from the 23rd through the 34th day, body weight returned to control value. When the drug was withdrawn, the dog promptly gained weight. After the single injection of the saluretic agent on the 78th day, the dog lost 0.7 kg of body weight within 24 hours. The attendant decrease in weight was maintained when the drug was administered orally until chlorothiazide therapy (5 mg/kg twice daily) was withdrawn on the 88th day. Thereafter, the dog began to gain weight under influence of the steroid. The ability of this agent to counteract the sodium and fluid retention induced by steroids has been confirmed by the repetition of this type of experiment in other dogs and by conventional renal clearance experiments in this species.

Chlorothiazide is an orally active saluretic (or diuretic) agent. Its predominant effect is to increase the excretion of sodium and chloride. Secondarily, it

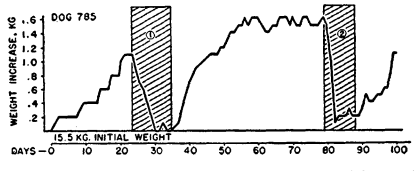



Fig. 1. Reversal by chlorothiazide of weight gain induced by the injection of 9α-fluorohydrocortisone and a high salt intake.