11448

Effects of Salts and Adrenal Cortical Extracts upon Toxicity of Drugs.

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Verzàr and his coworkers[1] in discussing the possible relationships between the adrenal cortex and fat, carbohydrate, and electrolyte metabolism, have postulated that the adrenal cortex controls a wide number of metabolic processes by regulating phosphorylation processes.

One of the primary arguments of these workers is that the effects of iodoacetate poisoning seemed to reproduce some of the symptoms of adrenal insufficiency, such as impaired selective intestinal absorption of glucose, muscular asthenia, steatorrhea, lowered body temperature, fluid loss by diarrhea, etc. These effects are ascribed to a specific inhibitory action of iodoacetate upon phosphorylation.

Laszt[2] has found that NaCl therapy antagonizes the iodoacetate effects in the intact rat, with respect to fatal toxicity, and impaired intestinal glucose absorption. This observation was offered as a possible explanation of some of the beneficial effects of NaCl therapy in adrenal insufficiency.

It appeared possible that this so-called "experimental adrenal insufficiency" produced by iodoacetate, could be explained on another basis than by specific inhibition of phosphorylations. This possibility was brought to our attention by the well known efficacy of NaCl, base, and fluid therapy in many toxic conditions, such as in mercury poisoning,[3] in which fluids are lost by emesis and diarrhea, and uremia ensues as a result of kidney damage. Such toxic agents could not be expected specifically to inhibit phosphorylations under the control of the adrenal cortex, although some of them may produce changes which have been termed symptoms of the "alarm reaction" by Selye and his coworkers.[4] For this reason, we have investigated the

1 Verzàr, F., *Die Funktion der Nebennierenrinde*, Basel, Benno Schwabe & Co., 1939; *Absorption from the Intestine*, Longmans, Green & Co., N. Y., 1936.

2 Laszt, L., *Nature*, 1939, **144**, 244.

3 Haskell, C. C., Carder, J. R., and Coffindaffer, R. S., *J. Am. Med. Assn.*, 1923, **81**, 448.

4 Selye, H., *Archiv. Internat. Pharm. Therdp.*, 1937, **55**, 431; *Am. J. Physiol.*, 1938, **123**, 758.

effects of NaCl, KCl, fluids, and adrenal cortical hormone upon rats poisoned with several non-specific toxic agents, including iodoacetate.

Four groups of 10 rats each were divided into 2 subgroups averaging ca 150 g per rat, each group receiving subcutaneously 50 and 80 mg per kg of sodium iodoacetate, respectively. This dosage is distinctly a lower level than the 100-120 mg level employed by Laszt. The rats were fasted and placed on the following *ad libitum* drinking fluids: (1) tap water, (2) 0.6% NaCl, 0.2% Na Citrate, (3) 0.2% KCl, and (4) tap water. Each group was given by stomach-tube 1 cc per sq decimeter of body surface of the following fluids: (1) tap water, (2) 1% NaCl, 0.1 N $NaHCO_3$ (ratio of 3:1) or 0.6% NaCl, 0.2% Na Citrate, (3) 1% KCl, and (4) tap water. The latter group was injected subcutaneously with Upjohn adrenal cortical extract, assaying 2.5 Cartland-Kuizenga rat units[5] per cc, at a level of 1 cc per rat at the time of administering iodoacetate, and again 4 hr later. The stomach-tubed fluids were given 4 hours before iodoacetate, at the same time as iodoacetate, and every four hours thereafter. Rectal temperatures were taken on all animals every 4 hours, and were seen to drop to approximately 95°F 4 hours after iodoacetate. Eight hours after the 50 mg level of iodoacetate, the NaCl group had nearly regained normal body temperature, with complete return in 12 hours; while all the other groups were still below 96°F at this time. All rats displayed muscular weakness progressing to collapse during the height of the effects of iodoacetate, and marked edema and inflammation at the site of injection, hemorrhagic diarrhea and hematuria. The NaCl group had a marked thirst for the NaCl-Na Citrate drinking fluid, and after 12 hours of fasting, the group which received the 80 mg level of iodoacetate had gained 37 g, showing a generalized edema.

The groups which received the 80 mg level all died within 8 hours except the NaCl group which survived, with return from 95° temperature to 98° within 20 hours. Potassium chloride decreased survival time, while cortin had no beneficial effects as compared with the controls on tap water. Another experiment, using 12 rats, was performed, in which survival times of cortin-treated and control iodoacetate-poisoned rats were compared for survival. More cortical extract was used than in the previous experiment (Wilson extract, assaying 1 d'Armour unit per 0.1 cc, 0.2 cc per 100 g body weight. Rats weighed av. 325 g), and was given at the time of administration of iodoacetate, and again 6 hours later. No effects

[5] Cartland, G. F., and Kuizenga, M. H., *J. Biol. Chem.*, 1936, **116**, 57.

on survival were obtained, thus confirming the previous result that cortin seems to have no beneficial effect on iodoacetate-poisoning.

Colchicine has been used by Leblond and Sagal[6] in a study of Selye's alarm reaction in rats. There are, however, no reasons to believe that this drug is a specific inhibitor of phosphorylations.

Preliminary experiments indicated that the minimal lethal dose of subcutaneously administered colchicine is less than 1 mg per kg, death being delayed. Food was offered *ad libitum* in the experiments with colchicine. Pathological symptoms were: collapse, weakness, lowered body temperature, hemorrhagic diarrhea and hematuria, loss of muscular tone, and poor muscular control.

Two levels of colchicine were given, 1 and 2 mg per kg, and essentially the same types of salt and fluid medication as described for the iodoacetate experiments. On the 2 mg level, in contrast to the iodoacetate results, KCl and cortin, as well as NaCl, exerted a beneficial effect as compared with the controls, with respect to body temperature, general activity, and survival. NaCl and cortin, especially the latter, exerted more marked effects. Fig. 1 illustrates the effect of NaCl and cortin on body temperature. It is noticed that the NaCl and especially the cortin groups nearly regain normal

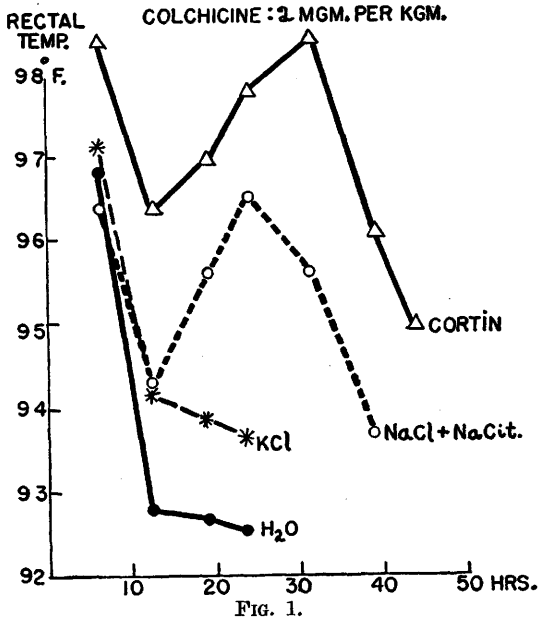

FIG. 1.
Effect of H_2O, NaCl-citrate, KCl, and Cortin Administration on Body Temperatures of Colchicine-Poisoned Rats.

6 Leblond, C. P., and Segal, G., *Compt. Rend. Soc. Biol., Paris*, 1938, **128**, 995.

temperatures, although the effects were not permanent at this level of colchicine. At the lower level of colchicine, the same results were obtained, except that several of the NaCl, KCl, and cortin animals survived indefinitely, whereas all the controls died.

Preliminary results demonstrated a minimal lethal dose of 10 mg per kg of subcutaneously administered $HgCl_2$ in rats. Death was markedly delayed, hence food was offered. Four groups of rats, averaging ca 240 g, were injected with 20 mg per kg of $HgCl_2$, thus a lethal dose. The groups were treated essentially as in the previous experiments, except that the hormone was given as subcutaneously administered desoxycorticosterone acetate in oil ("Doca") instead of cortical extract. One mg in oil was injected 0, 12, 24, 34, 43, 43 hr, and 2 mg at 51, 58, and 68 hr. The stomach-tubed fluids were administered at 24, 30, 35, 45, 60, 69, 80, 92, 104, 164, and 192 hr. Potassium chloride was definitely toxic to $HgCl_2$ poisoned rats, while "Doca" had no effect. The general symptoms were quite similar to those in iodoacetate poisoning except that death was delayed. All but one of the NaCl group had nearly regained normal weight within 2 weeks, and survived indefinitely. All others died; the KCl group first, followed by the "Doca" group, then the controls.

In summary, Fig. 2 shows the pooled results, with respect to survival times of rats poisoned with all 3 toxic agents. The top row of figures shows indefinite survival in all groups on the 50 mg level of

TOXIC AGENT & MGM. PER KGM	MEDICATION AND AV. HRS. SURVIVAL								MLD. MGM. PER KGM.
	NO. RATS	H_2O	NO. RATS	NaCl, NaCit.	NO. RATS	KCl	NO. RATS	CORTIN	
IAA 50	5	∞	5	∞*	5	∞	5	∞	>50 <80
IAA 80	11	8	5	∞*	5	5	8	7	
COLCH. 1	4	62	4	2-∞ 2-75	4	2-∞ 2-66	4	1-∞ 3-75	<1
COLCH. 2	7	20	4	28	4	30	4	43	
$HgCl_2$ 20	13	89	7	6-∞ 1-144	7	43	7	79**	10

*$NaCl + NaHCO_3$

** DESOXYCORTICOSTERONE ACETATE

FIG. 2.

Effect of H_2O, NaCl, KCl, and Cortin Administration on Survival of Iodoacetic-, Colchicine-, and $HgCl_2$-Poisoned Rats.

iodoacetate. The second row shows the marked life-maintaining effect of NaCl therapy in the case of lethal doses of iodoacetate, and lack of effect of cortin, while KCl seems somewhat toxic. The fourth row illustrates the beneficial effects of cortin in fatal colchicine poisoning. The last row of figures illustrates the toxicity of KCl, benefits of NaCl, and lack of effects of "Doca" in mercury poisoning. Temperature trends showed the same results as survival data, and in some cases revealed beneficial or detrimental effects much better throughout the entire study, except in the case of mercury poisoning.

Shorr, Barker, and Malam[7] questioned the specific inhibition of phosphorylation by iodoacetate during glucose oxidation. Wertheimer,[8] Klinghoffer,[9] Öhnell and Höber,[10] and Doty and Eaton,[11] have stated that iodoacetic acid has no effect upon intestinal absorption of sugar, salts, and amino acids by any specific inhibitory action, but does so if present in grossly pathological quantities, in which the toxic symptoms described in this paper would result. Most workers have used much larger doses than we have employed. Verzàr and his coworkers have used impaired intestinal glucose absorption as a criterion of adrenal insufficiency, and have extended reasoning obtained in such experiments to other experiments in which phosphorylation in general is claimed to become impaired in adrenalectomized animals. The data we have presented in addition to the quoted references seem to us to constitute an argument against comparing the non-specific pathology of iodoacetate poisoning with the symptoms seen in the adrenal insufficiency syndrome.

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7 Shorr, E., Barker, S. B., and Malam, M., *Science*, 1938, **87**, 168.

8 Wertheimer, E., *Archiv. ges. Physiol.*, 1933, **233**, 514.

9 Klinghoffer, K. A., *J. Biol. Chem.*, 1938, **126**, 201.

10 Öhnell, R., and Höber, R., *J. Cell. and Comp. Physiol.*, 1939, **13**, 161.

11 Doty, J. R., and Eaton, A. G., 52nd Proc. Am. Physiol. Soc., 1940, p. 50.