References and Notes

1. K. E. Wilzbach and A. R. Van Dyken, *U.S. Atomic Energy Comm. Doc.*, AECD-2998, Oct. 1950.
2. K. E. Wilzbach, L. Kaplan, and W. G. Brown, *Science* **118**, 522 (1953).
3. Tubing of Pyrex 1720 glass is available on special order from Corning Glass Works, Corning, N.Y. Quartz or Vycor tubing is also satisfactory.
4. A furnace, series 9ADL of the K. H. Huppert Co., Chicago, can be used, with a quartz liner, to heat eight tubes in one loading.
5. This temperature is obtained with a melt, formed by addition of liquid nitrogen, of toluene or di-*n*-butyl ether.
6. K. E. Wilzbach, A. R. Van Dyken, and L. Kaplan, *Anal. Chem.* **26**, 880 (1954).
7. Calibration with standard samples has established that the charge collected at 450 v in a Borkowski-type chamber filled to atmospheric pressure with carbon dioxide corresponds to 1.39×10^{-16} coulomb per disintegration of C^{14}.

8 July 1954.

Enhancement of Biological Activities of Corticosteroids by Substitution of Halogen Atoms in 9α Position

Grant W. Liddle, Maurice M. Pechet, Frederic C. Bartter

Section on Clinical Endocrinology, Clinic of General Medicine and Experimental Therapeutics, National Heart Institute, Bethesda, Maryland

The biological actions of hydrocortisone are readily distinguishable from those of desoxycorticosterone. Thus, while hydrocortisone has comparatively little sodium-retaining activity, it has until recently been the most potent known steroid in carbohydrate metabolism, in anti-inflammatory activity, and in depressing the number of circulating eosinophils. Desoxycorticosterone, on the other hand, while practically devoid of carbohydrate effects, anti-inflammatory activity, and eosinopenic activity, has until recently been the most potent known steroid in sodium-retaining activity. It has been of interest to us, therefore, that hydrocortisone, when it bears a fluorine or chlorine atom in 9α position, not only becomes more potent than hydrocortisone itself in carbohydrate and eosinopenic activity but also becomes more potent than desoxycorticosterone in sodium-retaining activity.

Fried and Sabo (*1, 2*) and Borman and Singer (*3*) have reported that substitution of either a fluorine or a chlorine atom in the 9α position of either cortisone or hydrocortisone results in enhancement of the activity of these corticosteroids as measured by glycogen deposition in the livers of fasting adrenalectomized rats.

We have studied, in adrenalectomized dogs, the comparative pharmacology of 9α-chlorocortisone acetate (chloro E Ac), 9α-chlorohydrocortisone acetate (chloro F Ac), 9α-fluorohydrocortisone acetate (fluoro F Ac), 9α-bromohydrocortisone acetate (bromo F Ac), cortisone (E), hydrocortisone (F), hydrocortisone acetate (F Ac), desoxycorticosterone (DOC), and desoxycorticosterone acetate (DOCA) (see Table 1). Several of these steroids have been studied, under a metabolic regimen, in patients with Addison's disease (*4*).

Effects on excretion of sodium and potassium. In adrenalectomized dogs, E and F, given intravenously in doses of 2 mg or less, had no appreciable effect on excretion of either sodium or potassium during the 4-hr period following administration. When given in doses of 4 to 20 mg, these steroids induced increases in excretion of both sodium and potassium during the ensuing 4 hr. In no dosage did E or F cause sodium retention. Chloro E Ac, chloro F Ac, and fluoro F Ac, on the other hand, resembled DOC in that each of these steroids in doses of 25 to 100 μg induced sodium retention and potassium loss. The magnitude of these responses was a direct function of dosage. Assays of these activities in adrenalectomized dogs indicated that chloro F Ac had 3.3 (1.9–5.2) (95 percent confidence limits) times the potency of equimolar doses of DOC, while chloro E Ac had 2.1 (1.2–3.8) and fluoro F Ac had 4.7 (2.4–9.2) times the potency of DOC. It was apparent that, insofar as these halogenated steroids caused acute sodium retention in adrenalectomized dogs, they differed qualitatively, as well as quantitatively, from their nonhalogenated analogs.

Effects on glomerular filtration rate. DOC apparently exerted its acute effects on electrolyte excretion by acting on renal tubular transport, since it failed to bring about any consistent changes in glomerular filtration rate (GFR) in doses up to 8 mg. Compounds

Table 1. Relative potencies of various steroids during 4-hr periods following intravenous administration to adrenalectomized dogs.

Steroid	Dose (μg)	Eosinopenia	GFR increase	Sodium retention	Potassium loss
Cortisone or Hydrocortisone Ac	100–2000	0	0	0	0
	4000–8000	+	+	– (Loss)	+
9α-Chlorocortisone Ac or 9α-Chlorohydrocortisone Ac	25–200	0	0	+	+
	500–4000	+	+	+ or –	+
9α-Fluoro-hydrocortisone Ac	25–100	0	0	+	+
	200–800	+	+	+ or –	+
9α-Bromo-hydrocortisone Ac	25–100			0	0
	100–400			0	+
Desoxycorticosterone	50–8000	0	0	+	+

E and F, in doses of 4 mg or more, and their halogenated derivatives, in doses of 0.5 mg or more, regularly induced increases in the GFR of adrenalectomized dogs; this effect became apparent during the second hour after administration of the steroids and was sustained througout the remaining collection periods. The sodium loss that occurred following administration of E and F during renal clearance studies was invariably associated with an increase in GFR. Whereas the halogenated steroids administered in small doses (which did not increase GFR) regularly caused acute retention of sodium, these same steroids administered in larger doses (which did increase GFR) frequently induced sodium loss. This paradox can be resolved by the assumption that all these steroids increase tubular reabsorption of sodium. This enhancement of sodium reabsorption, however, may be insufficient to result in a net conservation of sodium if the filtered load of sodium that is presented to the tubules is simultaneously increased.

Effects on circulating eosinophils. In adrenalectomized dogs, the decrease in circulating eosinophils was determined 4 hr following the intravenous administration of graded doses of various steroids, as a simple index of their "glucocorticoid" activity. When compared on an equimolar basis, fluoro F Ac was 20 (11–36) and chloro F Ac was 8 (5–13) times as active as F Ac.

Effects in Addison's disease. Studies carried out in two patients with Addison's disease indicated that the fluoro- and chloro-derivatives of F were considerably more effective in the treatment of this disease than were equimolar quantities of either F itself or DOC. In the longer term clinical studies, the enhanced potency of the halogenated steroids appeared to be even greater than that which was anticipated on the basis of the acute assays in animals. The symptoms of Addisonian crisis (nausea, vomiting, asthenia, and so forth) were corrected within 4 hr of the oral administration of either 0.5 mg of fluoro F Ac or 1.5 mg of chloro F Ac. Repetition of these doses every 4 hr resulted in progressive improvement in feelings of well-being, depression of circulating eosinophils, marked retention of sodium, and transient increases in potassium excretion. Withdrawal of the steroids was followed by a reversal of these effects.

Substitution of bromine. Bromo F Ac in doses of 25 to 100 μg had no effect on the excretion of sodium or potassium in adrenalectomized dogs, while in doses of 100 to 400 μg it induced some increase in the excretion of potassium but no consistent retention of sodium.

References and Notes

1. J. Fried and E. F. Sabo, *J. Am. Chem. Soc.* **75**, 2273 (1953).
2. ———, *ibid.* **76**, 1455 (1954).
3. A. Borman and F. M. Singer, *Federation Proc.* **13**, 185 (1954).

4. We wish to thank Joseph Fried of the Squibb Institute for Medical Research, Elmer Alpert of Merck and Co., and Robert Gaunt of Ciba Pharmaceutical Co. for generous supplies of the steroids employed in this study.

1 June 1954.

Serum Glutamic Oxaloacetic Transaminase Activity in Human Acute Transmural Myocardial Infarction

John S. LaDue, Felix Wróblewski, Arthur Karmen

Sloan-Kettering Institute and *Department of Medicine, Memorial Center, New York*

Glutamic oxaloacetic transaminase is widely distributed in animal tissues but is most concentrated in heart muscle (*1, 2*). This property led us to study its concentration in human serum following acute myocardial infarction.

The presence of this enzyme in human blood serum and whole blood hemolysates was previously demonstrated in our laboratory (*3*) using quantitative paper chromatographic analysis of the glutamate present after incubation of serum with aspartate and α-ketoglutarate. The chemical characteristics of the enzyme in serum were studied and found to be similar to those reported for animal tissues. The normal range of activity in human serums and hemolysates was established. The level was found to be elevated in certain disease states but notably so in two patients with acute transmural myocardial infarction.

These studies led to the development of a relatively rapid spectrophotometric assay of serum glutamic oxaloacetic transaminase activity and permitted the extension of our observations.

Transaminase activity is measured by adding serum to a substrate containing aspartate and α-ketoglutarate, which in the presence of malic dehydrogenare oxidizes DPNH to DPN. The resulting change in optical

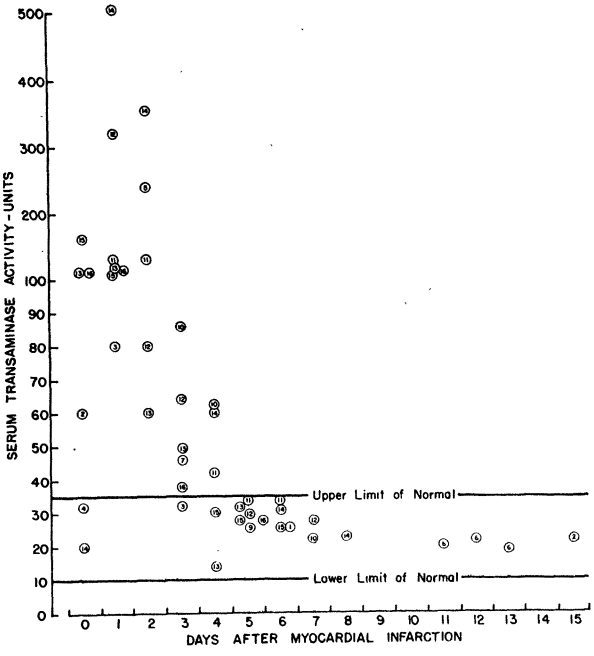

Fig. 1. Serum transaminase levels measured within a few hours to 15 days following acute myocardial infarction in 16 patients.