TABLE 1

INFLUENCE OF ACTH ON *in Vitro* INCORPORATION OF ACETATE-1-C^{14} INTO 17-HYDROXYCORTICOSTERONE

Acetate (μc/g gland tissue)	Compound F (17-hydroxycorticosterone)			
	Control		Added ACTH	
	Amount carrier F added (mg)	Specific radioactivity of isolated F (cpm/mg)	Amount carrier F added (mg)	Specific radioactivity of isolated F (cpm/mg)
13.5 (Hog)	0.5	46	0.5	92
83 (Cow)	2.0	102,000	2.0	200,000
94 (Bull)	2.15	3,370	2.15	7,350

ethanol, the extract was washed with ligroin, and the alcohol removed by distillation *in vacuo*. The remaining aqueous solution was extracted with methylene chloride, and the resulting extract was fractionated by chromatography on a column of silica gel. To each of the resulting corticosteroid fractions, a calculated weight of crystalline 17-hydroxycorticosterone (Table 1) was added as carrier. The diluted fractions were then chromatographed on filter paper strips in toluene-propylene glycol (*2*). The 17-hydroxycorticosterone zones were located, cut from the paper, eluted, and the specific radioactivity was determined (Table 1). Areas adjacent to the steroid zones were also eluted and were found to contain insignificant amounts of radioactivity. Characterization of the radioactive 17-hydroxycorticosterone eluted from the paper chromatograms was achieved by repeated chromatography on paper without decrease in specific radioactivity and by oxidation to adrenosterone. This oxidation of the 17-hydroxycorticosterone was carried out with chromic acid and gave rise to a ketosteroid which migrated on a paper chromatogram (ligroin-propylene-glycol system) as adrenosterone. The specific radioactivity of this steroid was of the same order as that of the 17-hydroxycorticosterone from which it was derived. The results of these experiments show that incorporation of C^{14} into 17-hydroxycorticosterone was increased twofold in the presence of ACTH.

Experiments were also done which demonstrated that added ACTH increased the output of formaldehydogenic steroids from adrenal slices. Corticoid extracts were chromatographed on silica gel, and the amounts of corticoids present were estimated by periodate oxidation of the appropriate column fractions, followed by determination of the liberated formaldehyde. Data from one such experiment given in Table 2 show that incubation with ACTH substantially increased the output of formaldehydogenic steroid by the slices. Liver slices, incubated and analyzed in the same fashion as the adrenal slices, showed no formaldehydogenic steroids before or after incubation.

TABLE 2

INFLUENCE OF ACTH ON *in Vitro* OUTPUT OF FORMALDEHYDOGENIC SUBSTANCES
(Values in μg formaldehydogenic steroid per g tissue)

Steroid initially present	Steroid at end of incubation						Output			
	Control			ACTH			Control		ACTH	
	No. vessels	Av	Range	No. vessels	Av	Range	Av	Range	Av	Range
44	4	71	62–78	4	126	107–148	27	18–34	82	63–104

In summary, the data indicated that the output of corticosteroids by adrenal cortex slices was substantially enhanced by the action of ACTH. The fact that ACTH stimulated the incorporation of C^{14} from acetate-1-C^{14} into 17-hydroxycorticosterone agrees with the finding of Hechter *et al.* (*3*) in perfused adrenals that ACTH accelerates the synthesis of corticosteroid hormones and not merely increases the rate of their release from the gland.

References

1. SAFFRAN, M., GRAD, B., and BAYLISS, M. J. *Federation Proc.*, **11**, 135 (1952); *Endocrinology*, **50**, 639 (1952).
2. ZAFFARONI, A., BURTON, R. B., and KANTMAN, E. H. *Science*, **111**, 6 (1950).
3. HECHTER, O., *et al. Recent Progr. Hormone Research*, **6**, 215 (1951).

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Concerning the Presence of Citrate in Commercial Crystallized Bovine Serum Albumin[1]

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It has become a widespread custom to use Armour's crystallized bovine serum albumin, without pretreatment, as a representative protein in the study of the theory of ion-protein binding. This preparation is obtained from citrated blood by the procedure of Cohn *et al.* (*1*). It appears to be generally assumed that all but an insignificant amount of the citrate is removed by the crystallization process. However, during the course of an investigation of the binding of beryllium by albumin, it has been discovered that the commercial albumin preparation contains a significantly large amount of a strong complexing agent. Considering the relative binding strengths of the various possible contaminants, it is believed that this anion is citrate and is present to the extent of at least 0.5 moles/mole protein.

Beryllium begins to hydrolyze and polymerize near pH 4.6, even in concentrations as low as 10^{-9} *M* (*2*).

[1] This publication is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y.

TABLE 1

DIFFUSIBILITY OF BERYLLIUM IN PRESENCE OF COMMERCIAL CRYSTALLINE BOVINE SERUM ALBUMIN, pH 7.5, 6° C*

Concentrations expressed in moles/liter × 10^4

Albumin in bag		Be outside of bag†	
		Found	If 100% diffusible
Untreated No. 1‡	2.8	0.81	0.91
" " "	2.8	1.53; 1.64	1.82
" " "	2.8	1.54	5.45
Untreated No. 2‡	2.8	0.73	1.82
" " "	2.8	2.04	5.45
Predialyzed No. 1	6.6	0.16	5.45
" " "	2.0–6.0	0.13	5.45
Buffer only		0.07	

* 10 ml solution prepared in buffer (.05 *M* barbiturate, .10 *M* NaCl) dialyzed *vs.* 10 ml buffer for 16 hr on Boerner oscillating platform shaker.

† Be concentration determined by radioactive isotope counting technique (*2*).

‡ No. 1 and No. 2 represent two different batches of Armour's crystalline bovine serum albumin.

Under the conditions of the present experiments (Table 1), the concentration of beryllium in barbiturate buffer at pH 7.5 and 6° C capable of diffusing through a cellophane bag is 7×10^{-6} *M*. When 10-ml solutions prepared by adding varying amounts of beryllium chloride (spiked with radioactive Be^7 isotope) to 2% Armour's crystallized bovine serum albumin in buffer were dialyzed *vs.* 10-ml volumes of buffer, the concentration of diffusible beryllium increased to about $1–2 \times 10^{-4}$ *M* (Table 1).

It was possible to decrease the amount of beryllium solubilizer associated with the protein to a very small, but still detectable, value by dialyzing 50 ml 8% crystalline albumin in a rotating cellophane bag *vs.* 20 liters buffer (0.025 *M* barbiturate, 0.125 *M* NaCl) two successive times at 6° C for 24 hr each time. As is seen in lines 6 and 7 of Table 1, 1.3×10^{-5} *M* beryllium is diffusible in the presence of this predialyzed preparation. A calculation from the data of lines 2, 7, and 8 of Table 1 $\{(0.13 - 0.07) \div [1.6 - (0.13 - 0.07)]\}$ shows that roughly 4% of the original contaminant was still present even after the dialysis treatment.

The seriousness of the presence of such a strong complexing agent must be considered in any theoretical study in which the commercial preparation is used, even if the identity of the contaminant is not positively known at present. The following results, however, suggest that the contaminant is probably citrate.

From a consideration of the crystallization process of the albumin, it seems that the only beryllium-solubilizing agents that could possibly be present in appreciable concentration are citrate, acetate, bicarbonate, and protein-decomposition products such as amino acids. It is evident from the data of Table 2 that, of these substances, only citrate could conceivably be in sufficient concentration to account for the diffusibility of beryllium in the presence of the unpredialyzed crystallized albumin.

TABLE 2

DIFFUSIBILITY OF BERYLLIUM IN PRESENCE OF SOLUBILIZING AGENTS POSSIBLY PRESENT IN COMMERCIAL CRYSTALLINE BOVINE SERUM ALBUMIN, pH 7.5; 6° C*

Concentrations expressed in moles/liter × 10^4

Added substance, total concentration†		Be outside of bag at equilibrium‡
Buffer only		0.07 ± .005 (5)§
Sodium citrate	0.91	1.85 ± .03 (2)
Sodium bicarbonate	100	0.11
Sodium bicarbonate	500	1.30
Sodium acetate	100 and 500	0.07 (2)
Amino acids: glycine, .0001 *M*; DL and L aspartic acid, and D and L glutamic acid, .0001 and .01 *M*; arginine, .001 and .01 *M*; cysteine, .002 *M*; serine, .001 *M*; histidine, .005 *M*; tryptophane, .005 *M*; tyrosine, saturated solution		0.07 ± .02 (16)

* 10 ml buffer (.05 *M* barbiturate, 0.10 *M* NaCl) containing 7.26×10^{-4} *M* beryllium plus added possible complexers dialyzed *vs.* 10 ml buffer for 16 hr on Boerner oscillating platform shaker.

† Initial concentration in bag ÷ 2.

‡ Be determined by radioactive isotope counting technique (*2*).

§ Parentheses indicate number of experiments averaged.

Since near pH 7.5 one citrate ion can bind a maximum of two beryllium atoms (*3*), one can calculate the probable minimum amount of citrate present in the crystalline protein. The data of Table 1 indicate that 2.8 μM albumin contains at least enough citrate to bind 3 μM beryllium—i.e., at least 1.5 μM citrate. Because some mass-action effect is to be expected, a citrate/albumin ratio greater than 0.5 seems to occur.

It is also of interest that a sample of Fraction V albumin contained a considerable amount of beryllium-solubilizing agent even after dialysis of 50 ml of a 25% solution in a rotating cellophane bag three successive times *vs.* 20 liters distilled water at 6° C each time. When a solution containing 1.1×10^{-3} *M* of this albumin and 6×10^{-5} *M* beryllium was dialyzed *vs.* an equal volume of buffer, all the beryllium remained in a diffusible state.

The significance of these results with respect to beryllium-protein binding will be discussed elsewhere.

References

1. COHN, E. J., HUGHES, W. L., JR., and WEARE, J. H. *J. Am. Chem. Soc.*, **69**, 1753 (1947).
2. FELDMAN, I., and HAVILL, J. R. *Ibid.*, **74**, 2337 (1952).
3. FELDMAN, I., *et al. Ibid.*, **73**, 4775 (1951).
4. SAFFRAN, M., and DENSTEDT, O. F. *J. Biol. Chem.*, **175**, 849 (1948).

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Added in proof: Citrate analyses, run in duplicate for each albumin sample, by the method of Saffran and Denstedt (*4*) indicate a minimum citrate/albumin ratio of 0.43 ± 0.04.