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INHIBITION OF THE SYNTHESIS OF PROTEIN IN INTACT ANIMASL BY ACETOXY*CYCLO*HEXIMIDE AND A METABOLIC DERANGEMENT CONCOMITANT WITH THIS BLOCKADE

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Abstract—The antibiotic, acetoxy*cyclo*heximide, markedly inhihits the incorporation of ^{14}C-amino acids into tissue proteins and Ehrlich ascites cells. Inhibition of inorganic ^{32}P incorporation into phosphoprotein is observed, whereas incorporation into acid-soluble compounds, RNA, and phospholipid is unaffected. The block of protein synthesis accompanies a syndrome characterized by vomiting, diarrhea, gradual hypotension, and death. The biochemical changes associated with this syndrome are progressive acidosis and elevation in the levels of plasma nonprotein N, urea N, amino acid N, potassium, inorganic phosphate and sulfate. The amount of plasma amino acid N increases at least three-fold within 3 hr of injection of drug; all the amino acids normally found in plasma are increased in concentrations. The antibiotics, actidione and puromycin, in doses several orders of magnitude greater than those of acetoxy*cyclo*heximide, also inhibit protein synthesis in the intact animal and induce a similar syndrome. The suggestion is made that this syndrome will prove to be typical of that induced by protein synthetic blockade in the intact animal.

A NUMBER of antibiotics inhibit the synthesis of protein in microorganisms; two, chloramphenicol and puromycin, are active both in bacterial[1, 2] and in mammalian cellular and subcellular preparations.[3–6] Puromycin was found recently to inhibit the incorporation of ^{14}C-amino acids into proteins of various organs in intact animals.[7, 8] The effect of another antibiotic, acetoxy*cyclo*heximide (E-73), on protein synthesis *in vivo* is reported in this communication.

Acetoxy*cyclo*heximide, 3-[2-(5-acetoxy-3,5-dimethyl-2-oxo*cyclo*hexyl)-2-hydroxyethyl]-glutarimide, was isolated from cultures of *Streptomyces albulus* and its structure characterized by Marsh and co-workers[9] and Rao and Cullen.[10, 11] The antibiotic was found to inhibit growth of transplanted tumors and to be toxic to yeast, mammalian cells in tissue culture, and intact animals.[9, 10] Investigations of the mechanism of toxic action of acetoxy*cyclo*heximide disclosed that the drug is the most potent inhibitor of protein synthesis in intact animals reported to this time. This effect and a metabolic derangement concomitant with the blockade are described in this paper. This induction of a similiar syndrome by other inhibitors of protein synthesis is also reported.

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MATERIALS AND METHODS

Animals used in these studies included female albino rabbits and female mongrel dogs, obtained from commercial suppliers; and mice derived from the Carworth CF-1 strain and rats derived from the Wistar strain, both randomly bred in our colonies. All animals were given routine laboratory diets and had food and water *ad lib.* until drug administration.

The Ehrlich ascites tumor was obtained through the courtesy of Dr. Marco Rabinovitz of the National Institutes of Health, Bethesda, Md., and was maintained by weekly transplants in mice.

Acetoxy*cyclo*heximide was supplied by the Charles Pfizer Co., Maywood, N.J. It was dissolved in sterile isotonic saline; the solution was stored at −20° and was thawed prior to use. Under these conditions, in the dosage range used for these studies, no loss of drug potency was observed over periods of 2 to 3 months. *Cyclo*heximide was purchased from the California Corporation for Biochemical Research and puromycin from Nutritional Biochemicals Corp. The algal hydrolysate was purchased from the New England Nuclear Corp., Boston, Mass., and had a specific activity of 0·2 to 1·0 mc/mg.

Measurement of the incorporation of ^{14}C-labeled algal hydrolysate into the proteins of the ascites tumor and of the livers of Ehrlich ascites tumor-bearing mice was carried out as follows. Acetoxy*cyclo*heximide or an equivalent volume of saline was injected intraperitoneally; 1 hr later, both control and treated mice received i.p. 1 μc of the labeled hydrolysate/g. Two hours after the injection of isotope, the mice were killed by cervical dislocation. The tumor cells were washed three times with chilled Krebs–Ringer phosphate buffer, then lysed with distilled water. The lysate was mixed with an equal volume of 10% TCA. Livers were minced and homogenized with a Teflon homogenizer in a solution of 0·5 M NaCl, 0·005 M $MgCl_2$, and 0·01 M Tris buffer, pH 7·6; liver protein was precipitated with an equal volume of 10% TCA. The protein was prepared for counting essentially by the method of Siekevitz.[12] Extractions were made in duplicate and samples were counted at "infinite thickness" in a Tracerlab TGC-14 Geiger–Müller counter. Measurement of the incorporation of the ^{14}C-labeled algal hydrolysate into rabbit liver proteins was carried out in the same fashion save that acetoxy*cyclo*heximide and isotope were administered via the intracardiac route. The animals were killed with Na Pentothal, and 6-g samples of liver tissue from at least three lobes were combined and extracted in duplicate.

In studies on the incorporation of inorganic ^{32}P into acid-soluble P, P-proteins, P-lipid, and nucleic acid fractions, 500 μg of acetoxy*cyclo*heximide/kg was administered intravenously to rabbits at zero time; 45 min later, 550 μc inorganic ^{32}P, dissolved in 0·5 ml isotonic saline was injected/kg, intramuscularly. The animals were sacrificed at 3 hr. Acid-soluble, lipid-soluble, RNA, and protein phosphate fractions of various tissues were isolated by the modification of the Schmidt–Thannhauser–Schneider procedure described by Volkin and Cohn[13] except that, after lipid extraction, nucleic acids were extracted with hot 10% NaCl, precipitated with ethanol and hydrolyzed as detailed by Davidson and Smellie.[14] Inorganic P and radioactivity measurements were made according to Ernster *et al.*[15] Aliquots from the final *iso*butanol–benzene solutions were used for determination of radioactivity and color.

Arterial pH, pCO_2 and HCO^-_3 measurements were made on left-heart blood by the electrometric technique of Siggaard-Anderson *et al.*,[16] using the Astrup type AME1b

meter (Radiometer, Copenhagen, Denmark). Plasma urea N, amino acid N, and phosphate were determined by standard clinical techniques of Natelson.[17] Non-protein N was assayed by Nesslerization. Na^+ and K^+ were measured by flame photometry. Ca^{2+} and Mg^{2+} were determined by tritration with EDTA according to the method of Grette.[18] Cl^- was determined by the technique of Schales and Schales.[19] Sulfate concentrations were measured with Dodgson and Spencer's modification of the benzidine method.[20] Arterial blood-lactate determinations were made by the Barker and Summerson technique as modified by Natelson.[17] Tissue extracts for free amino acid determinations were made with 1% picric acid as described by Tallan *et al.*[21] The automatic amino acid analyzer of Spackman *et al.*[22] was used to identify and quantitate the free amino acids in plasma.

RESULTS

The clinical syndrome

In a dosage range causing death within 8 to 36 hr, acetoxy*cyclo*heximide intoxication followed a predictable course. In the conscious animal, no effect was seen during the first hour. Vomiting and diarrhea often occurred within 3 hr. Subsequently, lethargy, asthenia, ataxia, stupor, coma and, finally, death were observed. In anesthetized dogs,* which received a dose of 2 to 4 mg/kg, no effect on blood pressure, pulse, or respiration was noted during the first hour. Thereafter, progressive hypotension developed, with the blood pressure eventually reaching 50 to 70 per cent of the control levels. Urine output ceased within 2 hr unless saline was infused. Death usually occurred by respiratory arrest or cardiac arrhythmia at a time when the blood pressure was still adequate to maintain life. Increasing the dosage in dogs to 20 times the LD_{100} did not shorten the interval between administration of the compound and death to less than 8 hr. No consistent histopathology was found following the acute toxic syndrome,† although in chronic toxicity studies in dogs hemmorhagic bronchopneumonia has been observed.[23]

Inhibition of the incorporation of ^{14}C-amino acids into proteins in intact animals

The effect of 5 mg of acetoxy*cyclo*heximide/kg on the uptake of ^{14}C-labeled amino acids by Ehrlich ascites tumor cells and by livers of the tumor-bearing mice is described in Table 1. As shown, the incorporation of labeled precursors into both cell types was inhibited approximately 95 per cent.

Table 1 also illustrates the effect of 500 μg of drug/kg on the incorporation of ^{14}C-labeled amino acids into liver protein in the intact rabbit. At this dose, which is approximately 7 $\times$ LD_{100} and is that used for the metabolic studies described in later sections of this paper, incorporation was inhibited more than 85 per cent.

Inhibition of the incorporation of ^{32}P into P-fractions in intact animals

The effects, *in vivo*, of acetoxy*cyclo*heximide (0·5 mg/kg) on the incorporation of inorganic P^{32} into P-fractions of various tissues of the rabbit are shown in Table 2. In liver, the antibiotic caused a significant decrease in the specific activity of ^{32}P-protein,

* Studies on anesthetized dogs were performed with the generous assistance of Drs. David McCurdy and M. Jerome Strong.

† Postmortem examinations on intoxicated animals were performed by Drs. Mitchell J. Rosenholtz and Brunildo A. Herrero.

TABLE 1. EFFECTS OF ACETOXY*CYCLO*HEXIMIDE ON INCORPORATION OF ^{14}C-AMINO ACIDS INTO THE PROTEINS OF INTACT ANIMALS*

Species	Tissue		Counts/min	Specific activity (%)
Mouse	Liver	Control	15,220	100
		Treated	720	5
Mouse	Ascites	Control	36,880	100
		Treated	1,600	4
Rabbit	Liver	Control	3,130	100
		Treated	390	12

* Data represent averages of two experiments. In the experiments with mice, the control and treated groups each consisted of two animals. The livers and ascites cells of the two animals were pooled and analyzed in duplicate. Five mg acetoxy*cyclo*heximide/kg was given i.p. 1 hr prior to the i.p. administration of 1 mc of a ^{14}C-labeled algal protein hydrolysate/kg. The animals were killed 2 hr later and the incorporated radioactivity measured as described in the text. In each experiment with rabbits, 6 g of liver was taken from the several lobes of each rabbit and processed in duplicate; 0·5 mg of acetoxy*cyclo*heximide/kg was administered 1 hr prior to the injection of the labeled amino acid mixture (0·1 mc/kg). Injections were made via the intracardiac route. Tissues were taken and processed as described in the text.

TABLE 2. EFFECTS OF ACETOXY*CYCLO*HEXIMIDE ON INCORPORATION OF ^{32}P INTO P-FRACTIONS OF RABBIT TISSUES*

Tissue	P-fraction	Specific activity (counts/min/μg P) Control	Treated	Sp. act. P-fraction/sp. act. P_i Control	Treated
Liver	Inorganic	37·3	64·3		
	Acid-soluble	15·0	31·5	0·40	0·49
	Protein	44·9	23·6	1·20	0·37
	Lipid	3·0	4·8	0·08	0·07
	RNA	0·8	1·8	0·02	0·03
Kidney	Inorganic	71·5	107·0		
	Acid-soluble	44·5	64·5	0·62	0·60
	Protein	34·9	27·0	0·49	0·25
	Lipid	3·5	7·3	0·05	0·07
	RNA	3·4	7·0	0·05	0·07
Appendix	Inorganic	63·0	59·3		
	Acid-soluble	33·5	32·8	0·53	0·55
	Protein	15·7	14·6	0·25	0·25
	Lipid	3·4	3·3	0·05	0·06
	RNA	6·7	3·9	0·11	0·07
Thymus	Inorganic	51·0	41·5		
	Acid-soluble	24·8	21·5	0·49	0·52
	Protein	7·4	7·5	0·15	0·18
	Lipid	3·7	4·9	0·07	0·12
	RNA	3·9	1·4	0·08	0·03

* Data represent averages of two experiments. Rabbits received 0·5 mg of acetoxy*cyclo*heximide/kg, i.v., at 0 time. At 45 min, 550 μc of ^{32}P/kg was injected i.m. Tissues were excised at 180 min and the different P-fractions were isolated as described in the text.

whereas the specific activities of inorganic ^{32}P, total acid-soluble^{32}P, ^{32}P-lipid, and ^{32}P-RNA were increased. The drug had no effect on the total quantity of these fractions with the possible exception of P-protein. This fraction was decreased slightly after injection of acetoxy*cyclo*heximide. Since the specific activity of inorganic ^{32}P in the treated animals was greater than that in the control animals, incorporation of inorganic P from this more highly labeled pool into the different fractions in the treated animals could result in an increase in the specific activities of these fractions without a concomitant change in the rate of synthesis of the fractions in the two groups of experimental animals. To compensate for this effect, the ratios of the specific activity of the various P-fractions to the specific activity of the inorganic P-fraction were calculated.* From these data, shown in Table 2, acetoxy*cyclo*heximide had little effect on the incorporation of ^{32}P into RNA, lipid, and acid-soluble fractions, whereas incorporation into P-protein was inhibited about 70 per cent. The magnitude of this inhibition of ^{32}P incorporation into liver protein is in good agreement with the 85 per cent inhibition found for the incorporation of ^{14}C-amino acids (Table 1).

The effects, *in vivo*, of the antibiotic on the incorporation of ^{32}P into P-fractions of the kidney were similar to those found in the liver. Table 2 shows that in kidney, ^{32}P incorporation into P-protein was inhibited over 50 per cent whereas incorporation into acid soluble-P, lipid-P, or RNA-P was not affected. In contrast, ^{32}P incorporation into P-protein of the appendix or the thymus was not inhibited in these experiments. It was noted, however, that acetoxy*cyclo*heximide caused a moderate inhibition of the incorporation of ^{32}P into the RNA fraction of these two tissues.

TABLE 3. ACUTE EFFECTS OF ACETOXY*CYCLO*HEXIMIDE ON NITROGEN METABOLISM*

		Hr after administration				
		0	1	3	5	6
		(mg/100 ml)				
Rabbit	Plasma nonprotein N	28		80	110	
	Plasma urea N	13		23	31	
	Plasma amino acid N	8		26	28	
Dog	Plasma nonprotein N	38	54	62	80	94
	Plasma urea N	22	30	38	49	57
	Plasma amino acid N	4	8	12	15	17

* Animals received acetoxy*cyclo*heximide i.v. (0·5 mg/kg).

Alterations in the plasma concentrations of nitrogenous substances

Because of the demonstrated action of acetoxy*cyclo*heximide on protein synthesis, plasma concentrations of various nitrogenous substances in treated animals were followed. Repeated studies in three species (dog, 500 μg/kg; rabbit, 500 μg/kg; rat, 350 μg/kg) showed a significant rise in levels of plasma nonprotein N, amino acid N, and urea N after administration of this compound. Table 3 illustrates typical results in the dog and the rabbit.

Additional information on the dramatic rise in plasma amino acid N was obtained by examining the amino acid patterns of plasma of rabbits after administration of

* This correction assumes that the rate of increase in the specific activity of inorganic P was linear throughout the experiment and that the same pool of inorganic P serves as the precursor for each fraction.

acetoxy*cyclo*heximide. In essence, these studies* revealed that the concentrations of all normal amino acids were increased. Although a precise delineation of these changes awaits further examination, no abnormal peaks were detected.

As noted in the description of the clinical syndrome, urine output in dogs dropped strikingly 2 hr after injection of 2 mg of the antibiotic/kg. However, the drug-induced rapid rise in nitrogenous substances in plasma is clearly distinguishable from a possible increase due to renal failure. In control experiments, rabbits were subjected to bilateral nephrectomy or ureteral ligations, and levels of plasma amino acid N and urea N were measured serially for 9 hr. The concentration of amino acid N gradually decreased from 8 to 5 mg/100 ml in these animals. In contrast, amino acid N increased from 8 to 26 mg/100 ml in 3 hr in rabbits treated with acetoxy*cyclo*heximide. A decrease in urinary excretion probably contributes to a portion of the drug-induced rise in plasma urea N. In the nephrectomized or ligatured rabbits, plasma urea N increased about 1 mg/100 ml per hr. On the other hand, the rise seen in rabbits injected with antibiotic (Table 3) was over three times this value. If one could extrapolate from the normal rate of urea excretion reported for the dog,[21] bilateral nephrectomy would not match the rapidity of the drug-induced rise in plasma urea N in this species, either.

*Relationship of liver and plasma free amino acid N levels following acetoxy*cyclo*heximide administration*

In order to test the possibility that the rise in the amino acid N in plasma was caused by leakage of free amino acids from tissue, liver and plasma levels were measured in the same animal. The results of these measurements in the rabbit, detailed in Table 4, show that liver free amino acid N increased approximately 25 per cent concurrent with the rise in plasma amino acid N. This observation is incompatible with the simple leakage hypothesis.

TABLE 4. COMPARISON OF THE EFFECT OF ACETOXY*CYCLO*HEXIMIDE ON LIVER AND PLASMA AMINO ACID N*

	Plasma amino acid N (mg/100 ml)		Liver amino acid N (mg/g wet wt.)
	0 hr	4 hr	4 hr
Control	7·0	6·3	2·6
Treated	7·2	24·5	3·4

* Rabbits received 0·5 mg of the antibiotic/kg, i.v. Each datum is the average of 6 rabbits. Values obtained in all experiments are within 10 per cent of the mean.

Alterations in blood pH and electrolytes

The observations that nitrogenous end products of protein catabolism accumulated as a result of acetoxy*cyclo*heximide intoxication suggested examinations of pH and of electrolyte concentrations in blood. Serial levels of Na^+, K^+, Cl^-, Mg^{2+}, Ca^{2+}, phosphate, and sulfate in plasma and arterial blood pH, pCO_2, and lactate were

* Dr. Earl Balis of the Sloan-Kettering Institute for Cancer Research, New York, generously performed the amino acid chromatography.

measured after the administration to rabbits of 500 μg i.v. of the antibiotic/kg. The following changes were consistently seen. The animals developed a progressive acidosis, with arterial pH values frequently dropping below 7·0. The pH decrease was concomitant with a drop in bicarbonate and an initial elevation in pCO_2. There were striking rises in phosphate and sulfate. A marked increase in K^+ was found. Terminally, blood lactate rose 4 to 6 mEq/l. The alterations observed in the levels of Na^+, Ca^{2+}, Mg^{2+}, and Cl^- were not significant. In Table 5 are shown the sequential changes in one of the animals.

Serial examinations of plasma electrolytes in dogs after injection of drug (500 μg/kg) showed changes in concentrations resembling those seen in the rabbit. Increases of as much as 2- to 3-fold within 6 hr in the levels of plasma inorganic phosphate and sulfate were particularly notable.

TABLE 5. ALTERATIONS IN RABBIT ARTERIAL BLOOD AND PLASMA AFTER ADMINISTRATION OF ACETOXY*CYCLO*HEXIMIDE*

	Hours after administration				
	0	3	6	9	11
pH	7·44	7·14	7·27	7·06	6·81
pCO_2 (mm Hg)	29·5	41·7	16·0	24·5	40·0
HCO_3 (mEq/L)	19·5	13·8	7·2	6·7	6·2
Na (mEq/L)	143	143	145	153	156
K (mEq/L)	4·2	6·0	6·9	9·1	10·2
Cl(mEq/L)	93	96	95	91	93
Inorg. SO_4 (mEq/L)	4·7	9·0	13·3	15·4	15·4
Inorg. PO_4 (mEq/L)	2·7	4·7	6·7	9·1	9·6
Mg (mEq/L)		2·2	1·9	3·1	3·7
Ca (mEq/L)		7·0	6·7	6·2	6·9

* Rabbit received 0·5 mg of the antibiotic/kg, i.v.

TABLE 6. EFFECTS OF *CYCLO*HEXIMIDE ON INCORPORATION OF ^{14}C-AMINO ACIDS INTO THE PROTEINS OF LIVER IN INTACT RABBITS*

	Counts/min	Specific activity (%)
Control	4,040	100
Treated	343	8

* Data represent averages of two experiments. In each experiment, 6 g of liver was taken from the several lobes of the rabbit, and extractions and analyses were made in duplicate. *Cyclo*heximide (50 mg/kg) was administered i.p. 1 hr prior to the i.p. injection of the labeled algal protein hydrolysate (0·1 mc/kg). After 2 hr the incorporated radioactivity was measured as described in the text.

Metabolic derangement produced by other inhibitors of protein synthesis

*Cyclo*heximide (Actidione*), less toxic to intact mammals but closely related to acetoxy*cyclo*heximide, has been shown to inhibit protein synthesis in yeast.[25, 26] The data in Table 6 demonstrate that it is a potent inhibitor of protein synthesis in an

* Trademark, Upjohn Co., Kalamazoo, Mach.

intact mammal also; 50 mg of *cyclo*heximide i.p./kg caused greater than 90 per cent inhibition of the incorporation of ^{14}C-amino acids into rabbit liver proteins.

If metabolic derangement induced by acetoxy*cyclo*heximide is related to a blockade of protein synthesis, it should also be produced by *cyclo*heximide in an appropriate dosage range. This indeed proves to be the case in every detail, as the data in Table 7 illustrate.

TABLE 7. METABOLIC ALTERATIONS IN THE RABBIT AFTER ADMINISTRATION OF A LETHAL DOSE OF *CYCLO*HEXIMIDE*

	Hr after administration			
	0	3	6	9
pH	7·46	7·24	7·14	6·80
pCO_2 (mm Hg)	33·3	33·0	35·0	24·5
HCO_3 (mEq/L)	22·8	13·6	11·4	3·9
Nonprotein N (mg/100 ml)	30		86	108
Urea N (mg/100 ml)	10	16	25	27
Amino acid N (mg/100 ml)	8	18	22	22
Na (mEq/L)	142	138	142	142
K (mEq/L	6·4	6·7	7·5	9·7
Cl (mEq/L)	102	107	107	101
Inorg. SO_4 (mEq/L)	4·2	5·4	8·1	
Inorg. PO_4 (mEq/L)	3·1	6·5	7·8	11·2

* Rabbit received 50 mg of *cyclo*heximide/kg, i.v.

TABLE 8. ALTERATIONS IN THE PLASMA OF THE RAT AFTER ADMINISTRATION OF PUROMYCIN*

	0 hr	6 hr
Urea N (mg/100 ml)	11	34
Amino acid N (mg/100 ml)	5	15
Na (mEq/L)	144	138
Cl (mEq/L)	112	109
K (mEq/L)	4·2	4·3
Inorg. PO_4 (mEq/L)	5·1	5·4
Inorg. SO_4 (mEq/L)	4·6	12·5

* Values represent the averages of individual determinations on five rats. Puromycin (375 mg/kg) was administered i.p. The dosage was 75 mg/kg per hr for 5 hr. Blood was drawn 1 hr after the final injection.

Puromycin, chemically unrelated to acetoxy*cyclo*heximide but known to inhibit protein synthesis in the intact animal,[7, 8] has now been found to produce alterations similar to those demonstrated for the *cyclo*heximide compounds. At a dose of puromycin causing 90 per cent inhibition of protein precursor incorporation into the liver of the mouse,[27] plasma urea N and amino acid N of mice increased severalfold. Changes in the levels of these nitrogenous substances as well as some plasma electrolytes after the administration of puromycin into rats are shown in Table 8.

DISCUSSION

The inhibition of protein synthesis by acetoxy*cyclo*heximide has been demonstrated by examining ^{14}C-amino acid and inorganic ^{32}P incorporation into proteins of ascites cells, liver of the mouse, and various tissues of the rabbit. Contrasting the present results with those reported by Hofert *et al.*,[27] 5 mg of acetoxy*cyclo*heximide/kg and a total dose of 300 mg of puromycin/kg give comparable inhibition of ^{14}C-amino acid incorporation in the mouse. Puromycin inhibition is more transient than that induced by acetoxy*cyclo*heximide; 90 per cent inhibition of protein precursor uptake over a 2-hr period was found only when puromycin was administered hourly before and during isotope incorporation.[27] On the other hand, a single injection of acetoxy*cyclo*heximide 1 hr before isotope administration caused a similar degree of inhibition. When 300 mg of puromycin/kg was given in a single injection at 0 time, inhibition of ^{14}C-amino acid incorporation fell from 90 per cent at 1 hr to 33 per cent at 3 hr.[27] Isotope incorporation and plasma N data in the rabbit, and plasma N levels in the rat and dog indicate that as little as 0·5 mg of acetoxy*cyclo*heximide/kg will cause greater than 85 per cent inhibition in these species throughout the interval between 1 and 3 hr. A comparison of the dosage-time–inhibition relationships for acetoxy*cyclo*heximide with those for puromycin suggests that acetoxy*cyclo*heximide is the most potent inhibitor of protein synthesis in intact animals reported to this time.

The data in Table 2 show that inorganic ^{32}P incorporation into lipids, acid-soluble nucleotides, and RNA continues at a normal rate in liver and kidney in spite of 70 per cent inhibition of incorporation into protein. These findings indicate that the inhibitory effects of acetoxy*cyclo*heximide are not general and suggest considerable specificity for protein synthetic mechanisms. The inhibitory effect of the drug on RNA synthesis in the thymus and appendix is not understood. It is particularly puzzling in view of the observation that incorporation of inorganic ^{32}P into the phosphoprotein fractions of these organs was unaffected by the drug. A similar inhibitory effect of puromycin on the incorporation of ^{32}P into RNA of rat thymus was noted by Gorski *et al.*[7]

If a block is placed on the synthesis of protein without a simultaneous brake being placed upon catabolic processes one would expect protein turnover to become net protein breakdown. The products of this breakdown as well as those from digested food may be used in other metabolic areas, be excreted, or accumulate. Mandelstam[28] showed that intra- and extra-cellular free amino acids accumulated in *Escherichia coli* cultures where protein synthesis had been blocked by chloramphenicol or by the absence of a specific essential nutrient. The data presented in this paper on the accumulation of amino acids in the plasma can best be explained on the basis of a disturbance of the equilibrium normally existing between protein synthesis and breakdown of tissue protein plus food protein. The data do not permit differentiation between gut and tissue as the source of the accumulating amino acids. Our experiments with nephrectomized and ligatured rabbits rule out an alternative explanation that the fall in urine output in the intoxicated animals is a major contributory factor to the increase in levels of plasma amino acid following drug administration. As noted above, a limited part of the rise in plasma urea after administration of acetoxy*cyclo*heximide may be due to a drop in urinary excretion.

The magnitude of the acidosis seen with the progression of the toxic syndrome is impressive. An obvious explanation could be that inorganic anions, sulfate and phosphate, accumulate as end stages of protein catabolism. The plasma buildup of

these anions presumably would be due both to the increase in their production by the tissues and to the drop in their urinary excretion. The latter may well be due to a combination of factors: i.e. hemoconcentration following loss of extracellular fluid into the gut, progressive fall in blood pressure and, conceivably, splanchnic vascular constriction. The rise in potassium is consistent with the breakdown of intracellular protein and the phenomenon of the intracellular–extracellular shift of potassium in the acidotic state.[29]

Although decreased renal function secondary to hypotension and renal ischemia may play a significant role in the toxic syndrome, hypotension alone cannot account for the speed with which the changes occur after administration of acetoxy*cyclo*heximide. In a series of experiments in which rabbits were bled of 30 to 40 per cent of their estimated blood volume (rabbits 2·0 to 2·5 kg, 55 to 65 ml total blood loss with an initial bleeding of 40 ml), little or no significant change in plasma urea N, amino acid N, or potassium levels was observed over a 9-hr period. Arterial blood pH changes were minor and generally tended toward alkalosis without a consistent pattern in pCO_2 and HCO_3^- alterations. The chemically-induced changes do, however, resemble in many ways those that can be produced by bilateral nephrectomy or ureteral ligation. Similarities include the rise in plasma nonprotein N, urea N, sulfate, phosphate, and potassium, and the progressive acidosis. The two syndromes differ primarily in the speed with which the biochemical changes occur and in the elevation of plasma amino acids observed in the drug intoxication. It is possible that this antibiotic intoxication could be used as a tool to investigate some of the catabolic aspects of the uremic syndrome as well as in studies of protein synthesis and turnover in the intact animal.

The studies with *cyclo*heximide and puromycin indicate that they can produce clinical and biochemical changes in rabbits, rats, and mice that are similar to those produced by acetoxy*cyclo*heximide. We suspect that this syndrome may be duplicable by many compounds that block protein synthesis in the intact animal. Indeed, tetracycline compounds, which inhibit bacterial protein synthesis,[1] have caused elevations of serum nonprotein N, potassium, and phosphate as well as acidosis in man and animals.[30–35] Chloramphenicol has been shown to inhibit protein synthesis in mammalian systems as well as in bacteria. Of particular interest is the lethal effect of the antibiotic in premature and newborn infants after several days' therapy at doses in excess of 100 mg/kg per day; in these infants, Burns *et al.*[36] noted that nonprotein N was elevated and, as their condition deteriorated, serum carbon dioxide fell and potassium rose. Although requiring high circulatory levels of drug over several days before they become manifest, the tetracycline and chloramphenicol effects are highly reminiscent of the syndrome induced by the *cyclo*heximide compounds described in this paper.

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