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EFFECTS OF COLCHICINE ON THE INCORPORATION OF NUCLEIC ACID PRECURSORS INTO RAT LIVER RIBOSENUCLEIC ACID*

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Abstract—Subcutaneous injection of 1·0–1·1 mg of colchicine/kg body weight into adult Sprague–Dawley rats 16–18 hr prior to the administration of radioactive nucleic acid precursors led to elevation over control values of the specific activities of the pyrimidines and purines of liver RNA. The greatest changes were in the specific activities of cytosine after orotic acid-6-^{14}C and uridine-2-^{14}C and of guanine after adenine-8-^{14}C and inosine-8-^{14}C. When xanthosine-8-^{14}C was given, colchicine treatment increased the specific activity of RNA guanine at least twofold. The results indicate that treatment of rats with colchicine may lead to enhancement of amination reactions in the liver, whereby a uracil nucleotide is converted to a cytosine nucleoide, and xanthosine-5′-phosphate is converted to guanosine-5′-phosphate. The change produced by colchicine in the liver concentration of free glutamine, the amino-group donor in these reactions, was of insufficient magnitude to account for the results.

COLCHICINE has long been known to exert chiefly two physiological effects, namely, arrest of dividing cells of plant and animal origin in metaphase and relief of pain in acute gout. The biochemical mechanisms of action of the drug remain obscure and are under investigation in this laboratory.

With regard to gout, the drug has been reported to exert little effect on the extent of renal excretion of uric acid or on serum uric acid concentrations in human subjects, and to leave unchanged the miscible pool of uric acid or the rate of turnover of this end product of purine metabolism. These metabolic aspects have been reviewed by Wyngaarden.[1]

Contrary to the findings in human subjects, some evidence has been obtained that colchicine may interfere with catabolic disposal of purines in the rat. Villela and co-workers[2,3] detected a 50%–80% decrease in the xanthine oxidase activity of the small intestine and liver and a rise in the activity of this enzyme in the serum of rats 1–3 days after i.p. injection of 1 mg drug/kg body weight.

These findings in the rat are suggestive of disturbances by colchicine in catabolic end-product formation from nucleic acid precursors. If this were the case, the possibility arises that utilization of such precursors for nucleic acid biosynthesis would be altered, and experiments were performed in our laboratory to test this hypothesis. The observations made during the course of the work prompted an analysis of the response of the levels of free amino acids in the liver to colchicine.

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MATERIALS AND METHODS

Isotopic compounds. Orotic acid-6-^{14}C was obtained from Nuclear-Chicago Corp., and uridine-2-^{14}C, adenine-8-^{14}C, inosine-8-^{14}C, and xanthosine-8-^{14}C from Schwarz BioResearch, Inc. The corresponding nonradioactive compounds were obtained from Calbiochem. The orotic acid-^{14}C was dissolved in 0·01 N NaOH and added to a solution of the unlabeled compound, which had been prepared by titrating an aqueous suspension with 0·1 N NaOH to slight alkali excess. After adjusting to pH 7 with dilute HCl, sufficient water was added to give final concentrations of 10 or 20 μmoles/ml. The xanthosine-8-^{14}C, furnished in 50% ethanol, pH 5·1, was supplemented with one fifth volume of 0·1 M Tris buffer, pH 8, and the solution was evaporated to dryness. A stock solution was prepared by dissolving the residue in aqueous xanthosine-^{12}C, 10 μmoles/ml, and adjusting to pH 7 with glacial acetic acid. The other stock solutions were prepared in water to provide 20 μmoles/ml of each compound.

Animals. Rats of the Sprague-Dawley strain were caged individually in the laboratory and maintained on Purina chow and tap water *ad libitum* for 30 hr prior to the start of the experiment. The animals were weighed before injecting colchicine or saline and before injecting a nucleic acid precursor. Food was withdrawn after injecting saline or drug, and tap water was given *ad libitum*. The weight ranges prior to the start of each experiment appear in the protocols.

Administration of colchicine and nucleic acid precursors. Colchicine (Abbott Laboratories, USP) in isotonic saline was injected s.c. at a level of 1·0–1·1 mg/kg body weight. Control animals received injections of saline. The volume of an injected solution approximated 0·5 ml the precise amount depending on the weight of the animal. Solutions of radioactive nucleic acid precursors were injected i.p. (approximately 2 ml per animal) 16 to 18 hr after the administration of colchicine or saline. After 5 hr the animals were anesthetized with ether and exsanguinated from the abdominal aorta.

Incorporation of nucleic acid precursors by liver slices. To determine the effect of colchicine on the ability of slices of rat liver to utilize orotic acid-6^{14}C for biosynthesis of the pyrimidines of RNA, the animals were handled in a manner similar to that used to study incorporation *in vivo*. Sixteen to 17 hr after they received colchicine or saline, the livers were removed and placed in ice-cold saline. Slices were prepared with a Stadie slicer and pooled in ice-cold saline either for the colchicine group or for the saline controls. To 20 ml of ice-cold preoxygenated Krebs–Ringer phosphate buffer,[4] pH 7·3, containing one half the indicated[4] amount of Ca^{2+} and 200 mg glucose/100 ml, was added 2·5 g of slices. Orotic acid-6-^{14}C was added to give a final concentration of 0·5 μmole/ml, and the mixtures were incubated with gentle rocking for 2·5 hr at 37° in an atmosphere of 100% oxygen.

Isolation of nucleic acid components. In experiments in which nucleic acid precursors were injected into the animals, the livers and other organs were removed and frozen on solid CO_2. The sodium nucleates were obtained by the method of Roll and Weliky[5] and digested with 1 N NaOH at 30° for 24 hr and separated into the DNA and the barium ribomononucleotides.[5] The barium ribomononucleotides were hydrolyzed to the free purines and pyrimidines with 72% $HClO_4$ for 1 hr in a boiling water bath.[6] The bases were then obtained as silver complexes and subsequently free from silver with HCl.[5] Aliquots of the acid solutions, sufficient to provide 5–10 μmoles of each base, were applied to sheets of Whatman 1 filter paper and subjected to descending

chromatography in duplicate using the *iso*-propanol–HCl solvent of Wyatt.[7] Standards of adenine, guanine, uracil, cytosine, and thymine were chromatographed with the samples. To provide suitable blanks a sheet of filter paper was hung from the same trough containing the sample sheet. After 20–24 hr of solvent migration, the sheets were dried at 30°, the bases located with the aid of u.v. light, and the areas cut from the paper. Corresponding blank areas were also removed. The paper was cut into small pieces and extracted with 3 portions of water, each for 30 min, at the temperature of a boiling water bath. The sample and corresponding blank solutions were evaporated to dryness to remove traces of HCl and the residues dissolved in water. Concentrations were determined by means of u.v. light spectrophotometry (we used published constants and corrected for the blanks). Blank values did not exceed 10% of the absorbance at the λ_{max} for each compound. The location of the λ_{max} and the spectrophotometric constants for each compound were in excellent agreement with published values. The area corresponding to thymine was also analyzed in order to determine the magnitude of contamination of the ribomononucleotides by deoxyribonucleic acid and was found to be negligible in all the experiments.

In the procedures with liver slices the vessel contents were chilled in an ice-bath after the incubation, 6 ml of ice-cold 25% trichloroacetic acid (TCA) was added, and the cold mixture blended in a high-speed mixer for 1 min. The supernatant obtained by brief centrifugation at 4° was discarded and the sediment washed 3 times with ice-cold 5% TCA. The sediment was then washed three times each with cold 80% ethanol, boiling ethanol, and boiling ethanol:ether, 3:1, twice with ether, and then dried in air. The mononucleotides of the RNA were liberated by a slightly modified Schmidt-Thannhauser procedure,[8] with 1 ml of 1 N NaOH for every 100 mg of residue and hydrolyzed for 24 hr at 30°. After neutralization in an ice bath with 0·25 volume of 6 N HCl and an equal volume of 5% TCA, the barium ribomononucleotides were isolated after centrifugation and the individual free bases isolated as described above.

Separation of subcellular fractions of liver. In one experiment the nuclei, cytoplasmic particles, and cytoplasmic supernate of the liver obtained at 2–4° by the methods of Hogeboom *et al.*,[9] Barnum *et al.*,[10] and Nygaard and Rusch,[11] were separated in order to examine the major RNA fractions. The livers were perfused with ice-cold 0·14 M saline and homogenized in 9 volumes (v/w) of 0·25 M sucrose–0·00018 M $CaCl_2$. The homogenate was passed through 4 layers of surgical gauze, the nuclei sedimented at 600 *g* for 10 min, washed with sucrose–$CaCl_2$, and again centrifuged. The nuclei were then dispersed in 0·25 M sucrose–0·00018 M $CaCl_2$, sedimented into a layer of 0·34 M sucrose–0·00018 M $CaCl_2$ at 600 *g* for 10 min, washed twice each with 2% citric acid and with 2% acetic acid, blended at high speed with 5% TCA, and washed twice with TCA.

One half of the cytoplasmic fraction (combined with the sucrose–$CaCl_2$ washings of the nuclei) was centrifuged in rotor no. 30 in the Spinco model L preparative ultracentrifuge for $3{\cdot}4 \times 10^6$ *g*-min[12] to obtain the particulate fraction (mitochondria plus microsomes), which was then homogenized briefly in 5% TCA. The insoluble material was collected in the centrifuge and washed twice with 5% TCA. The ultracentrifugal cytoplasmic supernate was adjusted to a concentration of 5% TCA and the insoluble material sedimented by brief centrifugation and washed twice with the TCA. A portion of the unfractionated cytoplasm was brought to the boiling point and supplemented with 2 volumes of ethanol and the precipitate collected.

All final sediments were subjected to the ethanol, ethanol–ether, and ether treatments as described above. The ribomononucleotides of RNA were obtained as the barium salts from the nuclei after hydrolysis with 1 N NaOH in the Schmidt–Thannhauser procedure and from the other fractions in the procedure used for the whole organs.

Measurement of radioactivity. The radioactivity of the isolated components was determined in an internal flow counter (Nuclear-Chicago Corp.) on "infinitely thin" samples (0·100–0·800 μmole) prepared in aluminum planchets. Sufficient background and sample counts were recorded until the standard counting error was 5% or less.[1]

Determination of the RNA content of the liver. The RNA content of the livers of animals treated with colchicine and of control animals was determined by the method of Albaum and Umbreit,[14] with adenosine-3′-phosphate as standard, after removal of acid-soluble nucleotides with cold 5% TCA.

Determination of free amino acids in liver. After administration of colchicine or saline the animals were anesthetized with ether, and in two experiments the livers were perfused with isotonic saline. In another experiment the animals were exsanguinated from the abdominal aorta without perfusion. The livers were removed and chilled in ice-cold saline, and the blotted wet weight was obtained. They were then homogenized in 4 volumes of ice-cold 5% TCA, the mixtures clarified by brief centrifugation at 4°, and the sediments washed twice with cold 5% TCA. The supernatant solutions were combined, the TCA removed by extraction with ether, and the aqueous solutions dried from the frozen state. Prior to analysis, the residues were dissolved in citrate buffer, pH 2·2, and a quantity representing one tenth of the TCA-soluble material of the liver subjected to quantitative ion-exchange chromatography for the determination of individual amino acids by the method of Spackman *et al.*[15] The glutamine–asparagine peak was resolved by refluxing in 6 N HCl for 18 hr, and the resultant glutamic and aspartic acids were determined as above.[15]

RESULTS

Effect of pretreatment of rats with colchicine on the conversion of orotic acid-6-^{14}C and adenine-8-^{14}C to RNA pyrimidines and purines of rat organs in vivo

Specific activities of the RNA pyrimidines of liver, kidney, and small intestine of control and colchicine-treated animals 5 hr after injection of orotic acid-6-^{14}C appear in Table 1. The greatest changes produced by colchicine were the increase of 2·6- and 2·8-fold in the activity of liver cytosine. The activity of liver uracil approached a twofold increase over the control values. The failure to observe the twofold increase in the specific activity of kidney cytosine in the second experiment and the small changes in the specific activities of the intestinal pyrimidines in both experiments prompted the decision to restrict the investigation to the liver. The data in Table 2 confirm the results of the prior experiments. Again, pretreatment with colchicine enhanced the specific activity of RNA cytosine to a greater extent than it did that of uracil.

The effect of colchicine was not limited to pyrimidine biosynthesis. The results of experiments with adenine-8-^{14}C indicate that colchicine produced a twofold greater incorporation into RNA adenine and a fivefold greater conversion to RNA guanine (Table 3).

Alterations produced by colchicine in what may be termned the patterns of the conversions of precursor orotic acid and adenine are discernible in Table 4, where the specific activity ratios of uracil/cytosine and adenine/guanine, obtained from the data of the first five experiments, are tabulated.

TABLE 1. CONVERSION OF OROTIC ACID-6-^{14}C TO RNA PYRIMIDINES IN THE ORGANS OF RATS TREATED WITH SALINE OR COLCHICINE

There were 6 male animals in each group. The ranges in body weight were 180–237 g in experiment 1 and 192–211 g in experiment 2. The amounts of colchicine injected were 1·0 mg and 1·1 mg/kg body weight respectively, 16 hr prior to injection of orotic acid ($1{\cdot}20 \times 10^5$ cpm/μmole) at levels of 0·20 and 0·19 mmole/kg body weight respectively. The animals were sacrificed 5 hr after receiving the orotic acid. The results are expressed as the specific activities of the isolated pyrimidines and as the ratios of the values observed after colchicine to those after saline.

Treatment	Liver		Kidney		Small intestine	
	Uracil (cpm/μmole)	Cytosine	Uracil (cpm/μmole)	Cytosine	Uracil (cpm/μmole)	Cytosine
Experiment 1						
Saline	3,080	1,240	3,330	1,210	1,030	243
Colchicine	5,020	3,180	2,930	1,220	714	184
Experiment 2						
Saline	1,990	833	2,120	675	809	174
Colchicine	4,150	2,340	3,210	1,270	730	219
Ratio (colchicine/saline)						
Experiment 1	1·6	2·6	0·88	1·0	0·69	0·76
Experiment 2	2·1	2·8	1·5	1·9	0·90	1·3

TABLE 2. CONVERSION OF OROTIC ACID-6-^{14}C TO RNA PYRIMIDINES OF LIVER FRACTIONS OF RATS TREATED WITH SALINE OR COLCHICINE

Colchicine was administered at 1·1 mg/kg body weight 16·5 hr before injection of orotic acid-6-^{14}C at 0·19 mmole/kg body weight after which the animals were sacrificed 5 hr later. There were 6 male animals per group, ranging in weight from 233 to 292 g prior to administration of drug or saline. Specific activity of the orotic acid was $1{\cdot}44 \times 10^5$ cpm/μmole. The results are expressed as the specific activities of the isolated pyrimidines and as the specific-activity ratios, colchicine/saline.

Fraction	Pyrimidine	Saline (cpm/μmole)	Colchicine (cpm/μmole)	Colchicine / Saline
Nuclei	Uracil	11,900	17,100	1·4
	Cytosine	5,540	11,600	2·1
Whole cytoplasm	Uracil	1,890	5,880	3·1
	Cytosine	776	3,810	4·9
Cytoplasmic particles	Uracil	1,510	5,020	3·3
	Cytosine	670	3,370	5·0
Cytoplasmic supernate	Uracil	2,850	7,380	2·6
	Cytosine	1,390	4,600	3·3

Effect of colchicine on the utilization of ribonucleosides for biosynthesis of RNA

The first step in the biosynthesis of RNA from orotic acid and adenine is condensation with 1-pyrophosphorylribose-5-phosphate to form orotidine-5′- phosphate and adenosine-5′phosphate respectively.[16,17] The increased utilization of the pyrimidine and purine precursors suggests that colchicine may stimulate these reactions.

To determine whether the effect of colchicine was limited to such reactions, the conversions of the nucleosides uridine-2-^{14}C, inosine-8-^{14}C, and xanthosine-8-^{14}C to the liver RNA bases were compared in control and drug-treated animals. There is strong evidence[18,21] that in the rat these conversions take place without cleavage of the nucleosidic bond. Primarily, two kinds of reactions are involved: (1) phosphorylation to nucleotides; and (2) substitution of a keto group by an amino group in a

TABLE 3. INCORPORATION OF ADENINE-8-^{14}C INTO RNA AND CONVERSION TO RNA GUANINE IN THE LIVER OF RATS TREATED WITH COLCHICINE OR SALINE

There were 4 male animals per group ranging in weight from 181–212 g and 4 female animals per group with a weight range of 240–285 g. The doses of colchicine were 1·0 and 1·1 mg/kg body weight and those of adenine ($7{\cdot}40 \times 10^{1}$ cpm/μmole) 0·17 and 0·15 mmole/kg body weight, respectively, administered 16 and 16·5 hr later. The animals were sacrificed 5 hr after receipt of the adenine.

Sex	Isolated compound	Saline (cpm/μmole)	Colchicine (cpm/μmole)	Colchicine / Saline
Male	Adenine	787	1,790	2·3
	Guanine	77	394	5·1
Female	Adenine	1,433	2,520	1·8
	Guanine	133	622	4·7

TABLE 4. PATTERNS OF INCORPORATION OF OROTIC ACID-6-^{14}C AND ADENINE-8-^{14}C INTO RAT LIVER RNA PYRIMIDINES AND PURINES RESPECTIVELY

Calculated from the specific activities appearing in Tables 1–3.

	Specific activity ratios			
	Uracil/Cytosine		Adenine/Guanine	
Precursor	Saline	Colchicine	Saline	Colchicine
Orotic acid	2·5	1·6		
Orotic acid	2·4	1·8		
Orotic acid	2·3*	1·5*		
Adenine			10·2	4·5
Adenine			10·8	4·1

* Average values obtained from the data in Table 2.

resultant uracil nucleotide (possibly uridine triphosphate) to produce a cytosine nucleotide (possibly cytidine triphosphate[22]) and replacement of keto groups by amino groups in inosinic acid and xanthylic acid to produce adenosine-5′-phosphate[23] and guanosine-5′-phosphate[24,25] respectively.

The specific activities of the liver RNA purines and pyrimidines after the injection of the ^{14}C-labeled ribonucleosides are presented in Table 5. With uridine as precursor there was little difference in the specific activity of uracil between the control and the drug-treated animals, but the specific activity of cytosine in the colchicine-treated

TABLE 5. CONVERSION OF RIBONUCLEOSIDES TO RNA COMPONENTS OF THE LIVERS OF RATS TREATED WITH SALINE OR COLCHICINE

There were 3 male animals per group. The dose of colchicine was 1·1 mg/kg body weight. The times after colchicine at which the nucleosides were injected were 16·5 hr for uridine and xanthosine, and 17·5, 17·5 and 18·0 hr for experiments 1, 2, and 3, respectively, with inosine. Sacrifice occurred 5 hr later.

Injected compound and specific activity (cpm/μmole)	Dose (mmole/kg)	Weight range of animals (g)	RNA Component: Saline Uracil (cpm/μmole)	Saline Cytosine (cpm/μmole)	Colchicine Uracil (cpm/μmole)	Colchicine Cytosine (cpm/μmole)	Colchicine/Saline Uracil	Colchicine/Saline Cytosine
Uridine-2-^{14}C $1·03 \times 10^5$								
Experiment 1	0·19	190–210	254	52	297	122	1·2	2·3
2	0·19	204–239	214	38	285	115	1·3	3·0
			Saline Adenine (cpm/μmole)	Saline Guanine (cpm/μmole)	Colchicine Adenine (cpm/μmole)	Colchicine Guanine (cpm/μmole)	Colchicine/Saline Adenine	Colchicine/Saline Guanine
Inosine-8-^{14}C $7·68 \times 10^4$								
Experiment 1	0·30	173–233	115	44	324	371	2·8	8·4
2	0·40	151–160	308	151	289	331	0·94	2·2
3	0·29	169–188	181	66	402	481	2·2	7·3
Xanthosine-8-^{14}C $1·41 \times 10^5$								
Experiment 1	0·13	178–205		123		257		2·1
2	0·13	207–224		100		288		2·9

animals was at least twice that of the control value. In the experiments with inosine-8-^{14}C at the body-weight level of 0·3 mmole/kg, the specific activity of the guanine was increased at least sevenfold over the control value and at the level of 0·4 mmole/kg, it was increased twofold. Where the greatest change in the guanine occurred there was, concomitantly, at least a twofold increase in the specific activity of the adenine of the drug-treated animals. With xanthosine-8-^{14}C as precursor, pretreatment with colchicine led to at least a twofold increase in the specific activity of guanine. Negligible activity was detected in the adenine in the experiments with xanthosine and, hence, no data for this purine are presented in Table 5.

The results obtained with the nucleosides indicate that colchicine probably augments reactions which involve nucleotide conversions beyond condensations with 1-pyrophosphorylribose-5-phosphate.

Conversion of orotic acid-6-^{14}C to RNA pyrimidines by liver slices in vitro

The incubation of liver slices from control and colchicine-treated rats with orotic acid-6-^{14}C resulted in the conversion of the precursor to RNA pyrimidines (Table 6).

TABLE 6. CONVERSION OF OROTIC ACID-6-^{14}C TO RNA PYRIMIDINES BY SLICES OF RAT LIVER

Three animals comprised each group in all the experiments. Females were used in experiment 4. The weight ranges in each experiment were: 1, 223–266 g; 2, 185–214 g; 3, 167–190 g; 4, 188–200 g. The dose of colchicine was 1·1 mg/kg body weight. Times of sacrifice after receipt of drug ranged from 16·5 to 17 hr from one experiment to the next. In each experiment portions of 2·5 g of slices of the pooled livers from each group were incubated in duplicate in 20 ml of Krebs–Ringer phosphate–glucose, pH 7·3, containing 0·5 μmole orotic acid-6-^{14}C/ml, $1{\cdot}62 \times 10^5$ cpm/μmole, for 2·5 hr at 37° in 100% O_2. The specific activities are the averages found for each pyridine isolated from the individual aliquots of slices.

Experiment	After saline		After colchicine		Colchicine/Saline	
	Uracil	Cytosine	Uracil	Cytosine	Uracil	Cytosine
	(cpm/μmole)		(cpm/μmole)			
1	570	179	865	359	1·5	2·0
2	630	176	971	401	1·5	2·3
3	638	173	935	339	1·5	2·0
4	550	112	1,002	387	1·8	3·5

As in the experiments *in vivo*, colchicine produced at least a twofold increase in the conversion of orotic acid to cytosine. This result demonstrates that the increased utilization *in vivo* is not attributable, most probably, to accelerated transport of the precursor from the intraperitoneal site of injection to the liver.

Liver RNA levels of colchicine-treated rats

This parameter was not exhaustively examined when little difference was found between the saline and colchicine groups in two experiments. In one experiment with 6 male animals (208–220 g), 17 hr after 1·1 mg colchicine/kg body weight, the range of RNA/g wet liver in individual animals, expressed as micromoles of adenosine-3′-phosphate, was 14·3–15·5 in the controls and 14·5–15·2 in the drug group. In the experiment where fractionation of the liver was performed (Table 2), the values for the homogenates were 16·5 and 17·2 for the control and drug groups respectively.

Changes in the liver content of free amino acids produced by colchicine

The greatest changes produced by colchicine in all the experiments were found in the specific activities of cytosine and guanine of the liver RNA. This finding points to the possibility that reactions involving substitutions in nucleotides of keto groups by amino groups are enhanced by colchicine. The source of the amino groups in cytidylic[22] and guanylic[24,25] acids is glutamine, and the amino group of adenylic acid arises from aspartic acid.[23] If one adopts as a working hypothesis the possibilities indicated by the isotope data, it becomes necessary to determine whether enhanced amination of nucleotides is a reflection of the production by colchicine of increased levels of the donors of the amino groups in the liver.

TABLE 7. CHANGES IN THE CONCENTRATIONS OF FREE AMINO ACIDS IN THE LIVERS OF RATS WHICH RECEIVED COLCHICINE

There were 2 males in each group. The weight ranges in each experiment were: 1, 176–178 g; 2, 193–203 g; 3, 169–194 g. The dose of colchicine was 1·1 mg/kg body weight, and the animals were sacrificed 17 hr later. In the first experiment the livers were removed after exsanguination. In the other experiments the livers were perfused with ice-cold 0·14 M saline after exsanguination. The liver of each animal was analyzed individually and the results averaged for each group.

Amino acid	Per cent increase from saline-treated animals Experiment		
	1	2	3
Aspartic	55	104	51
Glutamic	73	160	60
Glutamine	49*	24*	−17†
Asparagine			−10†
Threonine	37	47	56
Serine	83	70	60
Alanine	145	173	41
Glycine	11	6	15

* Glutamine plus asparagine.
† Decrease.

There is a suggestion (Table 7) that aspartic acid was elevated by at least 50% in the liver after colchicine. Glutamine which was found to be by far the preponderant member of the glutamine–asparagine peak in the ion-exchange profile, was somewhat elevated in two of the experiments and decreased slightly in the last. There is a suggestion that only the increase in aspartic acid would be consistent with the view that an essential metabolite is made more available for nucleotide synthesis.

It is of interest that threonine, serine, and alanine were also elevated and, paradoxically, that the level of glycine was scarcely affected.

DISCUSSION

There have been few studies of the effects of colchicine on nucleic acid biosynthesis in rodents. Clark and Stoerk[26] examined only those organs of the rat which had been recognized to be grossly affected by colchicine. At a dosage level and experimental timing similar to those used in the present experiments, they observed a marked decrease in the incorporation of inorganic phosphate-^{32}P into the DNA and RNA of

spleen, thymus, and lymph node. Skipper *et al.*[27] observed little change in the incorporation of the purine precursor formate-^{14}C into the combined DNA and RNA isolated from the pooled livers, small intestines, kidneys, testes, and spleens of mice. Any effect on liver RNA could have been masked by the pooling of the organs and of both nucleic acids. In mice, which had received 0·03 mg of colchicine, Kelly[28] found that one day later the incorporation of inorganic-^{32}P into liver DNA in 2 hr was tenfold greater than in the control livers, but reported no observations on the RNA. In the present experiments the DNA bases were considerably less radioactive than those of the RNA. The only reliable measurements obtainable on DNA with the methodology applied would be forthcoming from examination of the thymine after giving orotic acid-^{14}C or uridine-^{14}C. Small amounts of contamination of DNA by appreciably more radioactive RNA would obscure the precise activities of the DNA adenine, guanine, or cytosine. Nevertheless, thymine specific activities were too low to be of value. Under the experimental conditions, the principal effects were observed in the RNA.

The use in the present work of precursors highly specific for nucleic acid biosynthesis revealed an altered pattern of their utilization brought about by colchicine. All the isotope incorporation data are consistent with the idea that one biochemical effect of colchicine may be to increase the rate of amination of uracil-containing nucleotides and of those nucleotides containing hypoxanthine and xanthine. Increases in the liver concentration of the necessary amino acids did not consistently satisfy a change in substrate concentrations which could serve as a more immediate cause of the effect. A possible cause of the increased specific activities of the RNA components in the colchicine-treated animals would be reduction in the liver pool sizes of the 5′-phosphates of uridine, cytidine, adenosine, and guanosine. If the rates of conversion of the precursors, used in the present work, to these compounds were the same in the control and drug-treated liver, the specific activities of the mononucleotides available for RNA synthesis in the latter would be increased correspondingly. However, no significant decrease in the liver concentrations of the mononucleotides was observed by Wang *et al.*[29] 8 and 24 hr after adult male albino rats received 1 mg colchicine/kg body weight.

One is left with the impression that an increase in the liver nucleotide-aminating enzymes may occur as a result of administering colchicine. Although xanthine oxidase may be representative of some rat liver enzymes whose activities are decreased by the drug,[2,3] it does not follow that other enzymes may not be increased; at least one liver enzyme activity—alkaline phosphatase—was observed by Ebner and Strecker[30] to be increased after they gave colchicine to rats. It will be of interest to examine, for example, the influence of colchicine treatment on the well-defined uridine-5′-phosphate aminating system of rat liver described by Hurlbert and Kammen.[22]

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