adrenal cortex inhibitor (o, p-DDD) was capable of blocking the stimulatory effect of vit. D_3 on absorption of Ca^{45} by *in vivo* intestinal loops in rachitic chicks(9). However, there is no evidence from our results that the chick adrenal glands were selectively concentrating vit. D at least at 24 hours. The uptake by chick adrenals was small, only 0.08% being present in the rachitic and 0.05% in D_3-treated groups. The adrenal concentration (on wet weight basis) similarly was not distinctive, only 0.6% per gram for both groups.

Summary. The distribution of vit. D_3-4-C^{14} in rachitic and vit. D_3-treated chicks was assessed following oral intubation into chicks fed rachitogenic diet alone (rachitic) or treated with 6 IU/day of vit. D_3. At 24 hours, the chicks were sacrificed and the radioactive material in each tissue was extracted from saponified tissue, then partitioned into a nonpolar fraction (iso-octane) containing primarily vit. D_3 and a polar fraction (67% methanol) containing only breakdown products. Radioactivity was assayed in the ANSitron liquid scintillation counter using automatic external standardization for correction of counting efficiency.

Radioactivity was widely distributed in all tissues of the chicks with high uptake, on a concentration basis, by intestinal tract, liver, kidneys, adrenal glands, spleen and gall bladder and in serum. Muscle and pancreas were low in localized uptake. However, bone, muscle and skin each quantitatively accounted for a greater percentage than liver. From the relative proportion of activity in the polar partition phase (67% methanol) compared to that in the iso-octane partition phase, it can be inferred that the intestinal tract activity was mainly vit. D, in contrast to skin, spleen and gall bladder, muscle, and bone where considerable breakdown products were present.

The small intestines of the rachitic animals contained considerably more activity than corresponding tissue in the vit. D_3-treated chicks, whereas livers, ceca and large intestines contained less activity. Rachitic chicks excreted more activity than the vit. D-treated animals. Other tissues (muscle, bone, skin, adrenal glands) were quite similar in uptake in both groups.

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Received April 19, 1965. P.S.E.B.M., 1965, v120.

Temperature Acclimation Effects on Monkey Liver RIBO- and Deoxyribonucleic Acid and on Ribonucleic Acid of Other Organs.* (30436)

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In the squirrel monkey (*Saimiri sciurea*) it has been found(1), as in other animals(2-6), that there is a growth of internal organs in cold acclimation. The heart, liver, pancreas, and kidneys of the monkey grow in response

* Supported in part by USPHS Grant CA 4271-05 from Nat. Cancer Inst., N.I.H.; from the U. S. Army, Grant DA-49-193-MD-2558; and from the Kaiser Foundation, Grant 50.

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to cold and, conversely, decrease in size during heat acclimation. Since growth involves the synthesis of protein which is directed by ribose nucleic acid (RNA), one might anticipate that prolonged cold exposure would induce a net increase in RNA and that heat acclimation might be accompanied by a decrease in amount of RNA. Some organs, such as the cerebral hemispheres, show no growth changes whatsoever in either heat or cold acclimation (*i.e.*, no observable net change in protein synthesis), and one might hypothesize that in this organ there are no changes in RNA levels. In order to see if these changes do occur, assays of RNA were made on various organs of control, heat-, and cold-acclimated monkeys.

In addition, assays of liver DNA and calculations of RNA:DNA ratios were made to see if these change in cold or heat acclimation. If the ratio increases, then one might suspect, among other possibilities, that either RNA is broken down at a slower rate or possibly is being synthesized more rapidly, thus suggesting a more rapid rate of DNA transcription. Results of such pilot studies, therefore, should be of value for planning more definitive experiments to explore RNA metabolism in temperature acclimation.

Methods and materials. Adult male monkeys weighing between 700-950 g were obtained from Central America. They were kept in the animal colony room for 1 month, during which time they were dewormed with diathiazinine (Dizan) and given tuberculin tests. None had tuberculosis. Monkeys which had obvious physical defects or showed advanced senility were not used in these experiments. Preliminary studies as to the extreme temperatures which these monkeys tolerated were made(1), and it was decided to use acclimation temperatures as close to those as would permit survival. One group of 20 monkeys was subjected to 14-15°C, another to 38-39°C, and a third to normal animal colony room temperature of 23-25°C. The exposure time was 42 days. The monkeys were caged individually and given Domina monkey food pellets which contain approximately 30% protein, 50% carbohydrates, and 15-20% fat, plus supplementary vitamins. This diet was supplemented with fresh fruit daily (apples and bananas) and meal worm larvae. Water was available *ad libitum.*

At the end of the exposure time the monkeys were decapitated, and the tissues removed and weighed for the present study were the heart, liver, pancreas, kidneys, and cerebral hemispheres. Samples obtained from the same location in each organ were weighed, quick-frozen in liquid nitrogen, and stored in the freezer for further study.

For the assays of RNA and DNA the method of Dische, as modified by Schneider (7), was used. Proteins were assayed using the standard Folin-Ciocalteau method.

Results and discussion. The results are presented in Table I. The most striking change in RNA and protein content occurred in the pancreas during cold acclimation. Total protein increased approximately 50% and total RNA by about 60%; thus, there was more RNA per g protein. The fact that there was a less apparent increase in total protein than in RNA may only reflect the rapid secretion of proteins such as digestive enzymes and pancreatic polypeptide hormones. Since the cold-exposed monkeys eat more than controls, it would seem that there must be an enhancement of pancreatic (exocrine and endocrine) secretion to take care of increased digestive load and increased blood glucose content. Thus, the observed growth of the pancreas and its increased protein and RNA content are in harmony with the physiological requirements imposed.

Among the possibilities which come to mind are that the increase in total RNA could result either from a faster cellular production by a fixed amount of DNA (*i.e.*, faster DNA to RNA transcription) or from cell proliferation, which involved first DNA replication followed by a net increase in RNA per organ, but not per cell. There is also the possibility of a decreased intracellular RNAase activity imposed by changes in cellular milieu. At any rate, any of these changes suggest that cold acclimation may affect even the internal milieu of the nucleoplasm or the length of active life of the messenger or soluble RNA's. Levels of total DNA in the pancreas of these

TABLE I. Liver Total RNA and Total DNA and RNA:DNA Ratios, and Total RNA of Other Organs in Control, Cold-, and Heat-Acclimated Monkeys.

Tissue or organ	Total wt (g)	Total protein/organ (g)	μg DNA/g protein	Total DNA per organ (μg)	μg RNA/g protein	Total RNA per organ (μg)	RNA:DNA ratio
Liver:							
Cold	20.82 ±.3*	6.11 ±.2	776 ± 8	4570 ± 38	1245 ± 11	7610 ± 91	1.67 ±.2
Hot	15.47 ±.4	4.90 ±.1	730 ± 12	3590 ± 52	1345 ± 9	6620 ± 62	1.84 ±.1
Control	18.53 ±.3	5.23 ±.2	765 ± 11	3990 ± 44	1450 ± 13	7580 ± 70	1.90 ±.2
Pancreas:							
Cold	2.11 ±.02	.46 ±.002	—	—	1587 ± 13	642 ± 9	—
Hot	1.48 ±.01	.25 ±.001	—	—	1400 ± 16	352 ± 4	—
Control	1.63 ±.01	.29 ±.001	—	—	1378 ± 12	402 ± 5	—
Heart:							
Cold	3.54 ±.02	.66 ±.003	—	—	532 ± 4	352 ± 6	—
Hot	2.88 ±.02	.62 ±.003	—	—	404 ± 6	250 ± 5	—
Control	3.02 ±.02	.60 ±.003	—	—	460 ± 3	277 ± 6	—
Kidneys (pair):							
Cold	4.11 ±.03	.59 ±.002	—	—	1448 ± 15	857 ± 7	—
Hot	3.29 ±.03	.47 ±.002	—	—	1235 ± 14	585 ± 6	—
Control	3.52 ±.02	.47 ±.002	—	—	1340 ± 8	627 ± 7	—
Cerebral hemispheres:							
Cold	—	—	—	—	1020 ± 9	—	—
Hot	—	—	—	—	926 ± 10	—	—
Control	—	—	—	—	942 ± 8	—	—
Comparisons							
Liver	†C > W $p \leq .05$ W > H $p \leq .01$	C > W $p \leq .05$ W > H $p \leq .05$	C = W = H	C > W > H $p \leq .05$	C = W = H	C = W > H $p \leq .05$	C = W = H
Pancreas	C > W $p \leq .01$ W > H $p \leq .05$	C > W $p \leq .01$ W > H $p \leq .05$	—	—	C > W = H $p \leq .05$	C > W $p \leq .01$ W > H $p \leq .05$	—
Heart	C > W = H $p \leq .05$	C > W $p \leq .05$ H = I‡	—	—	C > H $p \leq .05$ W = I	C > W = H $p \leq .05$	—
Kidney	C > W $p \leq .01$ W > H $p \leq .05$	C > W = H $p \leq .05$	—	—	C = W = H	C > W = H $p \leq .05$	—
Cerebral	—	—	—	—	C = W = H	—	—

* ± Standard error of the mean.
† C = cold-acclimated; H = heat-acclimated; W = control.
‡ I = intermediate, not significantly different from either of the other two statistical populations being compared.

cold-acclimated monkeys and the timing of shifts in RNA and protein content remain to be investigated.

In the pancreas of the heat-acclimated animals there is a slight decrease in total RNA and protein, but RNA per g protein remains at control levels. Contrary to the situation in cold acclimation, in heat acclimation the monkeys consume less food than do the controls, and it would seem that requirements for pancreatic digestive enzymes and insulin should decrease. Thus, if less secretable protein is produced but also less secreted, the total protein level should not drop drastically. In actuality, it decreased only about 14%, as did RNA content. It appears, therefore, that heat acclimation results in a repressed pancreatic synthesis of RNA and/or an enhanced cellular RNA degradation. Another possibility is that there is a net loss of cells, which

would account for the lowered total RNA content.

The liver of the cold-acclimated animals showed a slight increase in weight accompanied by an increase in total DNA. However, no measurable increase in total RNA occurred, suggesting that RNA synthetic response of the liver in the cold was diminished compared to that of the pancreas. Since preliminary studies have shown no signs of increased numbers of multinucleate (*i.e.*, binucleate) cells, the small but significant total DNA increase probably represented some cell proliferation. The fact that the protein was elevated by 15%, as was the DNA, also indicated cell proliferation.

In the liver of heat-acclimated monkeys there was a slight decrease in total DNA, RNA, and protein of approximately 10, 12, and 6%, respectively; whereas decrease in weight was about 16%, possibly due to a loss of stored fat and/or carbohydrate or to dehydration. In neither the hot nor the cold experimental groups were the changes in liver RNA or DNA levels sufficient to yield significant differences from control values in RNA: DNA ratios.

Production of changes in kidney nucleic acids in cold acclimation has been shown in hamsters(8,9). Thus, in this organ, which grows by about 20% in cold-acclimated monkeys, it was not surprising that there was a significant increase in total RNA of approximately the same percentage. In heat acclimation there was no decrease in kidney protein nor in RNA per g weight, although as in the liver of the heat-acclimated animals there was a significant decrease in weight of the organ.

Although cold acclimation does not cause an increase in heart RNA per g protein, there was an elevation in total RNA and in total protein. Thus, cold acclimation induced a true growth of the heart in these primates, as has been previously reported for cold-acclimated rodents(3). Interestingly, the heart, which has very little synthetic functions, gave the lowest RNA per g protein of any of the organs investigated.

The cerebral hemispheres showed no significant changes in RNA levels per g protein, as might be anticipated, since the mg protein per g wet weight, the weight per volume, and the apparent volume remained constant.‡

Summary. Studies were made to determine the influence of cold and heat acclimation on the RNA content of the heart, liver, pancreas, kidneys, and cerebral hemispheres in the squirrel monkey *(Saimiri sciurea)*. Total DNA was assayed, and liver RNA:DNA ratios were calculated. The total amounts of RNA in the kidneys, heart, and pancreas were increased as a consequence of cold acclimation: in the kidneys and heart proportionally to the increase per g protein, but in the pancreas—the organ which showed greatest change in RNA content—there was an increase in RNA per g protein. Since greater amounts of food are consumed in the cold, necessitating enhanced insulin and digestive enzyme secretions, the outflow of these proteins probably prevents a percentage protein accumulation in the pancreas equal to that of RNA. Cold exposure produced a significant increase in total DNA and total protein in the liver which may be due to hepatic cell proliferation. However, there was no increase in total liver RNA which, among other possibilities, might indicate a suppression of DNA transcription or an enhanced RNAase activity. Heat acclimation resulted in lower total protein in the liver and pancreas with no alterations in the heart or kidney protein levels. None of these organs exhibited a decrease in RNA per g. However, a decrease in total RNA proportional to the decrease in protein content was seen in the liver and pancreas; whereas no change in total protein or in total RNA occurred in the heart or kidneys. There were no changes in cerebral RNA per g protein consequent to either heat or cold acclimation.

‡ Total weights of the cerebral hemispheres were not possible to obtain accurately because the demarcation between thalamic tissue and cerebral tissue was not clearly definable, and in order to avoid getting any thalamic tissue frequently portions of the roots of the cerebral hemispheres were left attached to the rest of the brain. Densities of the shallow bowl-shaped dissected cerebral hemispheres were determined by use of Archimedes Law.

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Received April 21, 1965. P.S.E.B.M., 1965, v120.

Brain Cholesterol X: Effect of Scheduling on the Sterol Lowering Capability of Methylphenidate (Ritalin).* (30437)

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Methylphenidate (phenyl-piperidyl-(2)-acetic acid methyl ester) is a compound which causes marked psychomotor stimulation. As a stimulant, it is classified somewhere between amphetamine and caffeine(1).

Previous reports from this laboratory(2-5) dealt with the effect of methylphenidate (Ritalin) on brain cholesterol metabolism, after chronic drug injection. The scheduling of drug administration in previous studies was not evaluated. To investigate further the problem of drug mechanism on brain cholesterol, a more systematic approach was attempted. The present report is a study of the effect of single and multiple drug injection on brain cholesterol level.

Experimental. The 295 male Swiss mice of Albino stock used in these studies were supplied by Stout Farms of Dexter, Mich. Animals representing age groups 5-6 weeks or 8-10 weeks were selected randomly within each age group, divided, and segregated into groups, each containing 5 mice. Food and water were administered *ad libitum*. Food was withheld for 24 hours prior to sacrifice. During the experiment, weights and daily intake of solid food and liquids were recorded. No significant change in these variables was measured in the drug groups.

A fresh stock saline solution of methylphenidate (1%) was made each week. This stock solution was diluted immediately before injection. Intraperitoneal injections of saline or drug were made with a volume of 0.5 ml. The smallest drug dose chosen (4 mg/kg) represents a level which significantly lowered brain cholesterol; larger doses (20 mg/kg) were not as effective. These drug levels represent respectively 1/35 and 1/7 of the usual LD_{50}.

Mice were killed by decapitation. Brain and liver were removed, washed, blotted dry, placed in test tubes, and quick frozen (acetone and dry ice). Tissues were stored in an ice chest (—30°C).

Separate tissue aliquots were taken for water and lipid content. The wet tissues were extracted with acetone:alcohol:ether (4:4:1). Solutions were filtered free of precipitated protein. Analyses of free and esterified cholesterol in the filtrate were made by precipitation with digitonin(6) or tomatine(7); by paper chromatography(8); or by gas-liquid chromatography(9). Details of precipitation methods have been reported(6,7). The chromatographic method was a modification of LaBarre *et al*(8). Strips of filter paper 1″ × 30″ were drawn through a 5% (v/v) so-

* This project was supported in part by USPHS Research Grant NB-02235 from Nat. Inst. of Neurol. Dis. and Blindness, Michigan Heart Assn., and Ciba Pharmaceutical Co.