ical synthesis, and as therapeutic agents for the treatment of tuberculosis, depression, and cancer. Besides the liver necrosis found in therapy with isoniazid and iproniazid (*4*), hydrazines are known to produce many other toxic responses including methemoglobinemia, hemolysis, fatty liver, mutagenesis, and carcinogenesis (*12*). Although no evidence has yet been found that isoniazid is carcinogenic in man (*13*), the possible risk of neoplasia after long-term therapy with such drugs needs continual monitoring. The possibility that certain other hydrazine drugs such as carbidopa and phenelzine might be hepatotoxic in man should also be considered, because these hydrazines are apparently metabolized in part by mechanisms similar to those described for acetylhydrazine and isopropylhydrazine (*14*).

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Cholesterogenesis: Derepression in Extrahepatic Tissues with 4-Aminopyrazolo[3,4-*d*]pyrimidine

Abstract. *Administration of 4-aminopyrazolo[3,4-*d*]pyrimidine decreased serum cholesterol levels in the rat to less than 5 milligrams per deciliter. Coincident with this change, there was a 2.1- to 16.0-fold increase in the rate of sterol synthesis in seven extrahepatic tissues. This suggests that cholesterol carried in serum lipoproteins plays a major role in regulating sterol synthesis in many extrahepatic tissues.*

While all mammalian tissues that have been tested synthesize sterol in vitro, the highest rates of synthesis are usually found in liver and small intestine (*1*). Studies in vivo show these same two organs to be the major, if not the only, sources for circulating serum cholesterol (*2*). The rate of sterol synthesis in these tissues is responsive to a variety of control mechanisms: cholesterogenesis in the liver, for example, is modulated by such factors as stress, fasting, light-cycling, and variation in the amount of bile acid present in the animal (*3*). In addition, the rate of hepatic cholesterol synthesis varies inversely with the cholesterol content of the diet. This last control mechanism is mediated through the selective uptake by the liver of cholesterol carried in remnants of intestinal lipoproteins, and provides an important means for coupling the rate of endogenous sterol synthesis to the rate of intestinal absorption of exogenous, dietary cholesterol (*4*). Similarly, the rate of cholesterogenesis in the intestine also is responsive to such manipulations as fasting and alterations in the enterohepatic circulation of bile acids (*5*).

In contrast, sterol synthesis in essentially all other tissues in animals such as the rat and nonhuman primate occurs at very low rates and is not altered by such variables as light-cycling, fasting, bile acid pool size, or dietary cholesterol intake. However, in all of these situations the concentrations of lipoprotein-cholesterol in the serum are relatively constant. In view of the recent work in tissue culture showing that cholesterol carried in low density lipoproteins suppresses cholesterogenesis in the fibroblast (*6*), the possibility exists that sterol synthesis in one or more of the extrahepatic tissues also is suppressed by one of the major serum lipoprotein fractions.

To examine this possibility, we took advantage of the observation of Henderson and of Shiff *et al.* (*7*) that administration of the adenine analog 4-aminopyrazolo[3,4-*d*]pyrimidine (APP) apparently inhibits hepatic secretion of lipoproteins and causes a marked decrease in serum cholesterol levels. Using this analog, we have shown that cholesterol synthesis increases in at least seven extrahepatic tissues when the concentration of cholesterol in the serum is suppressed below 5 mg dl^{-1}.

Female, Sprague-Dawley rats initially weighing 190 to 210 g were subjected to light-cycling (12 hours of darkness and 12 hours of light) for 2 weeks. The light phase of the cycle began at 1500 hours while the dark phase began at 0300 hours. The animals were placed in individual metabolic cages and injected with either 0.9 percent NaCl solution (controls) or APP dissolved in 0.9 percent NaCl solution at a concentration of 5 mg ml^{-1} (experimental animals). The exact schedule for the injections is given in the legends to Table 1 and Fig. 1. During this treatment period each control animal was pair-fed with an animal receiving APP. To dissolve the APP (Sigma Chemical) it was necessary to titrate the solution to a *p*H of approximately 1.5 to 2.0 and then back-titrate to a *p*H of 4.0 just before giving the intraperitoneal injections. At the completion of the treatment period the animals were killed at 0900 hours, the mid-dark point of the light cycle, and various tissues were removed and immediately chilled in cold 0.9 percent NaCl solution. Tissue slices 0.8 mm thick were prepared and 300-mg (wet weight) portions were placed in 25-ml Erlenmeyer flasks containing 2.0 ml of oxygenated Krebs bicarbonate buffer (*p*H 7.4), 16 μmole of sodium acetate, and 10 mc of ^3H_2O. One set of flasks was incubated for 90 minutes at 37°C in a metabolic shaker set at 120 cycles per minute. A duplicate set of flasks was packed in ice and incubated with shaking under identical conditions at 0°C. At the end of the incubation period, 6 ml of alcoholic potassium hydroxide solution was added to each flask, and the tissues were saponified on a steam bath. Sterols were extracted with petroleum ether and precipitated as the digitonide. The radioactivity in the digitonin-precipitable sterols from the flasks incubated at 0°C was subtracted from the radioactivity in the digitonin-precipitable sterols obtained in the corresponding tissues incubated at 37°C. This difference was used to calculate the nanomoles of ^3H_2O incorporated into dig-

itonin-precipitable sterols per gram of tissue (wet weight) per hour.

The overall rate of sterol synthesis in any tissue can be estimated by using a variety of isotopically labeled precursors such as glucose, acetate, pyruvate, or octanoate. However, such substrates enter the cholesterogenic pathway through acetyl coenzyme A (acetyl CoA), and we have previously shown that significant dilution of the specific activity of the intracellular acetyl CoA pool occurs, at least in the hepatocyte (*8*). This problem can be circumvented in the liver by using an isotopically labeled substrate such as octanoate and correcting for intracellular dilution of the specific activity in the precursor pool; however, such a method cannot be employed in many of the extrahepatic tissues. Alternatively, one can measure the activity of β-hydroxy-β-methylglutaryl CoA reductase (HMG CoA reductase; E.C. 1.1.1.34) which has been shown generally to reflect the overall rate of cholesterol synthesis in tissues such as the intestine and liver. The activity of this rate-limiting enzyme has not been established in all the extrahepatic tissues, however, and we have reported at least one instance in which there is major dissociation of HMG CoA reductase activity from the overall rate of cholesterol synthesis (*9*). For the studies reported here, therefore, we measured the rate of incorporation of ^3H_2O into sterols under conditions where the rate of sterol synthesis was driven by the presence of a relatively high concentration of unlabeled acetate. Such a method measures the overall rate of sterol synthesis but is not subject to the criticism of significant and variable dilution of the specific activity in the intracellular precursor pool (*10*).

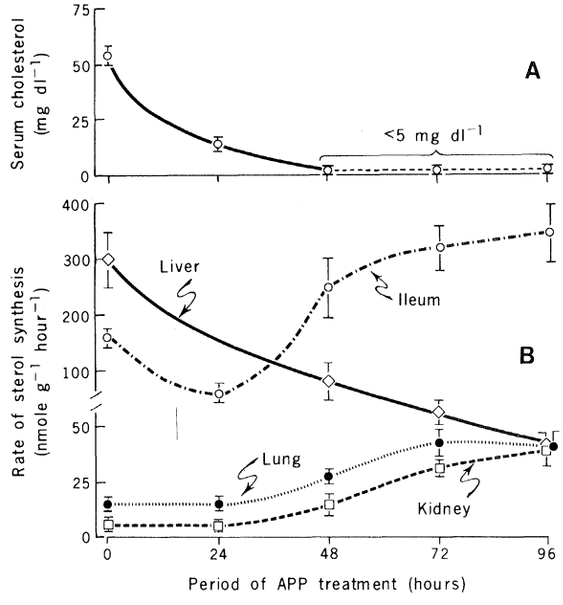

Fig. 1. Time course for the derepression of sterol synthesis in three extrahepatic tissues. Rats initially weighing 190 to 210 g were subjected to light-cycling for 2 weeks. Animals were then administered APP intraperitoneally (50 mg/kg, in 0.9 percent NaCl, *p*H 4.0) each morning at 0900 hours, the mid-dark point of the light cycle, and groups were killed every 24 hours thereafter for 4 days. (A) The total serum cholesterol level after different periods of treatment with APP. (B) The rates of incorporation of ^3H_2O into digitonin-precipitable sterols in tissue slices prepared from liver, terminal ileum, lung, and kidney at the same time intervals. Each point in (A) and (B) represents the mean ± standard error (S.E.) for four to five animals in each group.

First we examined the time course for the effect of APP on serum cholesterol levels and rates of sterol synthesis in several tissues as illustrated in Fig. 1. As reported (*7*), the serum cholesterol level fell more than 90 percent within 48 hours and remained at less than 5 mg dl^{-1} with continued APP administration between 48 and 96 hours. Concomitant with this fall in serum cholesterol there was a significant increase in sterol synthesis in the ileum, lung, and kidney but a progressive fall in the rate of ^3H_2O incorporation into digitonin-precipitable sterols by the liver. However, these animals ate only approximately 3 g of diet during the first 24 hours and nothing thereafter. Thus, there was a significant fasting effect superimposed on any effect in sterol synthesis induced by APP administration. Sterol synthesis in the liver is known to be very sensitive to fasting, while deprivation has relatively little effect on the extrahepatic tissues. This fact may explain why hepatic cholesterogenesis declined while synthesis in the other tissues markedly increased.

The increased rates of sterol synthesis became apparent at 48 hours and essentially remained at maximum values between 72 and 96 hours (Fig. 1). Thus, we chose the 96-hour time point to examine the effect of APP administration on sterol synthesis in 13 tissues of the rat. The data, shown in Table 1, illustrate the differences in rates of ^3H_2O incorporation into digitonin-precipitable sterols in tissues from animals injected with APP on four successive days and pair-fed control animals injected with 0.9 percent NaCl solution. Seven extrahepatic tissues showed significant (2.1- to 16.0-fold) increases in sterol synthesis: these tissues included the jejunum, ileum, colon, lung, kidney, ovary, and adrenal. In contrast, liver, pancreas, spleen, skin, and cardiac and striated muscle manifested no alterations in rates of sterol synthesis.

Four major points regarding these data warrant emphasis. First, the fact that there is an apparent inverse relation between the serum cholesterol level and

Table 1. The effect of APP administration for 96 hours on rates of sterol synthesis in 13 tissues of the rat. Groups of rats initially weighing 190 to 210 g were subjected to light-cycling for 2 weeks. Control animals were then injected intraperitoneally with 2.0 ml of 0.9 percent NaCl solution (*p*H 4.0) while the experimental group received APP (50 mg/kg, in an equivalent volume of 0.9 percent NaCl, *p*H 4.0) each morning at 0900 hours, the mid-dark point of the light cycle. These injections were repeated on four successive days during which time the rats were maintained in individual metabolic cages, and the control animals were pair-fed with the experimental group. At the end of this time the animals were killed at 0900 hours and the various tissues were assayed for their ability to synthesize digitonin-precipitable sterols from ^3H_2O. Mean values ± 1 S.E. are shown for data derived from four to seven animals in each group. The ratios of the rates of sterol synthesis in tissues from the APP-treated animals (E) to the corresponding control values (C) are shown in the third column; NS indicates that the two values were not significantly different at the $P < .05$ level according to Student's *t*-test.

Tissue	^3H_2O incorporation (nmole g^{-1} $hour^{-1}$)		
	Control animals	APP-treated animals	E/C
Liver	62.3 ± 14.0	42.6 ± 9.0	NS
Jejunum	66.7 ± 7.9	363.6 ± 42.0	5.4
Ileum	33.9 ± 5.5	354.9 ± 56.4	10.5
Colon	41.7 ± 10.5	134.0 ± 15.8	3.2
Pancreas	14.1 ± 12.0	13.6 ± 10.4	NS
Lung	4.4 ± 2.0	40.1 ± 7.0	9.1
Kidney	5.5 ± 2.0	39.4 ± 7.6	7.2
Spleen	19.6 ± 6.2	21.5 ± 5.0	NS
Adrenal	39.0 ± 5.8	623.9 ± 204.5	16.0
Ovary	420.2 ± 54.4	901.2 ± 53.6	2.1
Skin	46.3 ± 9.0	50.3 ± 11.6	NS
Heart muscle	9.3 ± 3.6	5.5 ± 2.6	NS
Striated muscle	1.0 ± 0.3	4.0 ± 2.3	NS

the rates of sterol synthesis in a number of extrahepatic tissues is consistent with the view that sterol synthesis in these organs is controlled by cholesterol carried in one of the serum lipoprotein fractions. Presumably, this mechanism is similar or identical to that described in the isolated fibroblast where low density lipoproteins have been shown to control the level of HMG CoA reductase (*6*). Second, it is of interest that only seven of the twelve extrahepatic tissues tested in this study manifested enhanced sterol synthesis (*11*). It is not known whether the remaining tissues would also have shown increased rates of synthesis if the period of observation has been more prolonged. Alternatively, it is conceivable that these tissues are subject to a different control mechanism. Third, it is impressive that the rate of sterol synthesis in the derepressed small intestine actually exceeded the rate in the liver of fed animals, that is, approximately 300 nmole per gram of tissue per hour. In contrast, in the other responsive tissues such as lung and kidney the observed rates of synthesis were still low relative to those in the gastrointestinal tract and liver. This finding again emphasizes the quantitative importance of these two organs to net sterol balance in the body. Fourth, cholesterogenesis in the liver is clearly regulated either directly or indirectly by remnants of chylomicrons, bile acids, and various hormones. Whether the liver also is regulated by serum lipoproteins to any significant degree cannot be determined on the basis of these studies because of the profound fasting effect that is undoubtedly present in the animals treated with APP for 4 days.

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Calcium Influx Requirement for Human Neutrophil Chemotaxis: Inhibition by Lanthanum Chloride

Abstract. *Calcium fluxes of human neutrophils measured in the presence of chemotactically active serum showed a marked stimulation of calcium-45 uptake from the media. Chemotactically inactive serum did not cause an influx of calcium. The magnitude of the calcium influx due to activated serum is sufficient to trigger contractile systems previously described in muscle cells. Lanthanum chloride inhibited the chemotactic response of human neutrophils to activated serum. Lanthanum in concentrations that suppressed chemotaxis also inhibited the calcium influx caused by activated serum. The data support a direct role of calcium influx in chemotaxis of neutrophils.*

The ability of a cell to translate a chemical gradient into directional motility, the phenomenon of chemotaxis, has interested cell biologists for many years (*1*). Chemotaxis of neutrophils has been demonstrated in vitro and the nature of the attractant characterized (*2, 3*). However the intermediary steps coupling the chemotactic agent to oriented motion remain essentially unknown. Colchicine and other antitubulins inhibit chemotaxis (*4*) and divalent cations appear to be required for neutrophil motility (*5*). Isolation of actomyosin-like proteins from both guinea pig and horse neutrophils (*6*) has raised the question of whether calcium activation of a contractile mechanism is involved in the directional movement of neutrophils. To answer this we measured the relationship between the in vitro chemotactic response and calcium fluxes of neutrophils exposed to activated serum. Lanthanum ion (La^{3+}), which inhibits transmembrane calcium movement, was employed to determine the role of calcium movement in chemotaxis.

The depressant effect of lanthanum on isolated frog heart contractions was demonstrated as early as 1910 (*7*). Since that time the specific antagonism of calcium binding by lanthanum has been elucidated (*8*). Smooth muscle is a contractile system requiring calcium and is quite sensitive to inhibition by lanthanum. The calcium influx required to sustain contraction of smooth muscle cells can be selectively blocked by concentrations of lanthanum around 0.1 m*M*. Millimolar concentrations of lanthanum are further capable of inhibiting calcium efflux in smooth muscle (*9*). Lesseps (*10*) demonstrated by electron microscopy the inability of lanthanum to penetrate embryonic chick cells. This suggests that lanthanum is selectively effective at the plasma membrane.

Employing a modification of the Boyden technique (*2*), we investigated the chemotactic responsiveness of isolated neutrophils to activated serum in the presence of La^{3+}. Neutrophils were collected from normal volunteers and isolated by sedimentation of blood in 3 percent dextran, followed by hypotonic lysis of contaminating red cells. The leukocyte preparation contained at least 85 percent neutrophils and less than 15 percent mononuclear cells. The cells were resuspended in Gey's medium and washed in a balanced salt solution (BSS) containing 135 m*M* NaCl, 4.5 m*M* KCl, dextrose (1 g/liter), 10 m*M* Hepes buffer (*p*H 7.2), 1.3 m*M* $MgCl_2$, and 1.5 m*M* $CaCl_2$. The final concentration of cells was adjusted to 2.0×10^6 neutrophils per milliliter. Lanthanum was added in the form of $LaCl_3$. Serum was obtained from normal volunteers, activated by incubation with endotoxin, and employed in the concentration that resulted in maximum chemotactic response (3 percent by volume in BSS). Samples in chemotaxis chambers were incubated in triplicate for 90 minutes, at which time the filters were removed, fixed, and stained, and the neutrophils that migrated to the lower surface were counted in 20 high-power fields.

Figure 1 demonstrates the effect of La^{3+} on the chemotactic response of neutrophils to activated serum (AS). Cell