nonspecific, i.e., it can process autologous antigens for allogeneic lymphocytes.

Summary. Suspensions of pure lymphocytes did not interact in mixed cultures; the reaction could be restored by the addition of purified monocytes or of unfractionated leukocyte suspensions in which lymphocytes had been rendered unreactive by X-irradiation or mitomycin. These findings suggest that the monocyte is essential to the mixed leukocyte culture reaction.

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Catabolism of Adenine Nucleotides by the Isolated Perfused Rat Heart* (32614)

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Some evidence exists that adenosine, a known coronary dilator, is involved in the regulation of coronary blood flow. Berne (1) found that perfused cat hearts released inosine and hypoxanthine, the degradation products of adenosine, into the perfusion medium under hypoxic conditions. From such findings he suggested that during periods of decreased oxygen tension or decreased coronary blood flow, heart muscle nucleotides were broken down to adenosine which diffused out of the cardiac cell, reached the coronaries via the interstitial fluid, and produced arteriolar dilation. Following this, deamination of adenosine by adenosine deaminase would yield inactive inosine. Since that time, considerable evidence has accumulated that adenosine is produced by cardiac tissue during anoxia. Richman and Wyborny (2) found that adenosine could be detected in perfusates of rabbit hearts treated with 8-azaguanine to inhibit adenosine deaminase and with dinitrophenol to uncouple oxidative phosphorylation. Moreover adenosine has been shown to appear in rat and rabbit ventricular tissue after short periods of ischemia (3, 4) and is present in coronary sinus blood of the ischemic dog heart (5). Baer *et al.* (6) have isolated a 5′-nucleotidase from heart muscle which dephosphorylates adenosine 5′-phosphate (5′-AMP) to adenosine. Adenosine deaminase was also isolated from ventricular tissue (6), and thus the enzymatic machinery for formation and degradation of adenosine is present in the myocardium. In the model of Berne (1), it was suggested that adenosine was produced in the myocardial cells and reached the coronary arterioles via the interstitial fluid. We have considered the possibility that 5′-AMP may be dephosphorylated to adenosine directly within the smooth muscle cells of the coronary vasculature. Some evidence that this might occur has been provided by Williamson and Di Pietro (7), who found that various 5′-nucleotides and other phosphate esters were dephosphorylated when continuously recycled through perfused rat hearts. Similar findings were reported by Hoffman and Okita (8)

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using guinea pig hearts. This report shows that 5′-AMP and adenosine triphosphate (ATP) are rapidly converted to adenosine and inosine during a single passage through perfused rat hearts.

Materials and Methods. Hearts from female Wistar rats were perfused in a nonrecirculating system using Tyrode's solution at 37°C equilibrated with 95% O_2, 5% CO_2. Radioactive substrates were injected through a small polyethylene catheter (void volume 0.03 ml) inserted into the perfusion line and opening into the tip of the cannula to which the aorta was attached. Routinely the rate of injection was in doses of 0.1 ml delivered over 5 sec. A starting tension of 5 gm was applied to each heart and contractile performance was monitored with a Statham force displacement transducer attached to a Grass polygraph. Separation of radioactive products was performed by paper chromatography of aliquots of perfusion fluid after addition of the appropriate carrier. Ultraviolet absorbing spots were cut out, placed in 15 ml toluene containing 4 gm of 2,5-diphenyloxazole and 50 mg of 1,4-bis[2-(5-phenyloxazolyl)] benzene, and counted in a scintillation counter. For solutions containing low levels of activity, the nucleotides and nucleosides were first adsorbed on Norit A, followed by elution with 20% pyridine, and the solution was concentrated by freeze-drying. When nucleotide content of tissue was examined, the hearts were frozen by crushing between metal clamps pre-chilled in liquid N_2. Frozen tissue was extracted in 10 volumes of 0.3 *N* perchloric acid; nucleotides and nucleosides were then adsorbed on Norit A and eluted as described above. Paper chromatography was performed in the following systems: (A) *n*-propanol, NH_4OH, water, 6:3:1; (B) *n*-butanol, glacial acetic acid, water, 5:2:3; and (C) water adjusted to pH 10 with ammonia.

Results. In preliminary experiments, hearts were perfused in a recirculating apparatus in which the perfusion fluid (25 ml) was recycled through the heart. When the perfusion fluid contained 0.8×10^{-4} *M* 5′-AMP, it was shown that this nucleotide was completely degraded within a 5-min perfusion period. Adenosine and inosine were the products. Radioactive 5′-AMP added to the perfusate after the experiment remained intact over a 2-hour period, and therefore dephosphorylation did not result from enzymes leached out of the heart. Because of this rapid degradation of 5′-AMP, it was reasoned that significant breakdown should occur during a single passage through the heart. When various nucleotides and nucleosides were injected into an open-flow perfusion system as described in *"Materials and Methods,"* a characteristic profile of radioactive distribution with time was observed in the perfusate. A *main* peak occurred up to 10 sec (measured from the time of beginning of injection) followed by an exponential decline up to 3 min (*tail* portion). This is shown for inosine and 5′-AMP in Table I. It can be seen that for inosine and 5′-AMP the *main* peak contained 67 and 63%, respectively, of the total radioactivity, whereas the *tail* portion contained 31 and 27%, respectively. Small amounts of radioactivity remained in the tissue after 3 min.

TABLE I. Distribution of Radioactivity Following Injection of Inosine and 5′-AMP.[a]

Substrate	Total activity (%)		
	Main portion	Tail portion	Tissue after 3 min
Inosine	67	31	2
5′-AMP	63	27	10

[a] Two hearts were used for each substrate and were frozen after 3 min for extraction of tissue nucleotides. The *main* portion of the perfusate was collected up to 10 sec from the time of beginning of the injection. The *tail* portion was collected between 10 sec and 3 min. 0.1 ml of 0.1 m*M* solutions were injected.

Analysis of purine derivatives within both the *main* and *tail* portions of the perfusion profile following the injections of inosine, 5′-AMP, adenosine, and ATP is shown in Table II. When adenosine was injected, virtually all of the radioactivity in the *main* portion appeared as adenosine, showing that it had gone through the heart with only 7% being deaminated. However, in the *tail* portion 58% appeared as inosine. When 5′-AMP was injected it was rapidly degraded as evidenced by the fact that 54% of the radioactivity in the *main* peak appeared as adenosine and only

TABLE II. Distribution of Label in Various Compounds Following Injection of Radioactive Substrates.[a]

Substrate injected		Radioactivity in perfusate (%)				
		ATP	5′-AMP	Inosine	Adenosine	X
Inosine	Main	—	—	98	—	2
	Tail	—	2	36	5	57
Adenosine	Main	—	—	7	92	1
	Tail	—	2	58	12	28
5′-AMP	Main	—	31	15	54	—
	Tail	—	6	13	10	70
ATP	Main	10	52	11	23	4
	Tail	2	9	9	15	65

[a] The values given are the mean of two injections of substrate into each of two hearts. Main and tail portions of the perfusate are as described in Table I, and injections consisted of 0.1 ml of 0.1 m*M* substrate. Separation of products was effected using both chromatographic solvents A and B.

38% was accounted for as 5′-AMP. Inosine accounted for 15% of the radioactivity in this portion of the perfusion profile. The ATP was extensively degraded; in the main peak, 5′-AMP accounted for 52% of the radioactivity, adenosine for 23%, and inosine for 11%. Radioactivity in the *tail* portion of the profile in each case contained large amounts of an unknown compound (X) (Table II). This compound has not been identified. Chromatographic analysis in solvents A, B, and C revealed that it was not adenine, xanthosine, xanthine, guanosine, allantoin, or uric acid.

Dephosphorylation of 5′-AMP could have been due to 5′-nucleotidase activity or to a nonspecific phosphatase. To examine this, 2′(3′)-AMP (mixed isomers) was also injected and radioactive materials in the perfusate were analyzed. Unreacted substrate accounted for 94% of the label in the *main* peak. This would indicate that the action is quite specific for 5′-AMP and point to the involvement of 5′-nucleotidase rather than phosphatase activity.

Discussion. It is clear that ATP and 5′-AMP are rapidly converted to adenosine and inosine during a single passage through perfused hearts. A question arises as to the locale of the enzymes which effect these catabolic changes. Most of the radioactivity appeared in the perfusate within 10 sec from the beginning of the injection (*main* peak). This was followed by an exponential washout of radioactivity lasting up to 3 min (*tail* portion). It is generally accepted that nucleoside phosphates do not readily enter cells from the extracellular space, that is, they do not readily cross cellular membranes. It would seem that most of the degradative processes occurring during the release of the *main* peak of radioactivity must be effected by enzymes within the vascular bed. More protracted changes during the exponential washout period of perfusion (*tail* portion) may result from uptake of metabolite or degradative products such as adenosine by cardiac cells, followed by release of adenosine, inosine, or the unknown compound back into the perfusion fluid. It would seem reasonable to suggest that adenosine triphosphatase and 5′-nucleotidase must exist within coronary vascular cells and be located so that their active sites are accessible to substrate at least from the extracellular lumen side of the membrane. Whether the same enzymes could effect the intracellular formation of adenosine, or whether this would depend on separate intracellular enzymes, is not established from the present experiments. We feel that there is a need for a clearer understanding of the precise site of adenosine formation in cardiac tissue. If enzymatic processes in the vascular bed are involved, it may be they respond more immediately to change in blood

oxygen tension than their myocardial counterparts and could, therefore, exert a finer control in regulation of coronary blood flow. We have shown (6) that the 5'-nucleotidase from rat myocardium is strongly inhibited by low concentrations of ATP (K_i 1.83 $\times$ 10^{-6} M). It was suggested that during oxygen sufficiency, the enzyme may be kept in restraint by virtue of high intracellular ATP concentrations. During hypoxia, when ATP levels decline, adenosine formation would facilitated by removal of inhibition. Possible similarities between the myocardial enzyme and the present activity in coronary vasculature are clearly of interest.

It has been assumed that the coronary dilator action of ATP and 5'-AMP is due to their prior conversion to adenosine (9, 10), which readily crosses cell membranes. The rapid conversion of ATP and 5'-AMP to adenosine by perfused hearts as described here would lend strong support to this idea.

Summary. ATP and 5'-AMP were extensively degraded to adenosine and inosine during a single passage through rat hearts perfused in an open-flow system. It is suggested that enzymes which convert adenine nucleotides to adenosine exist within, or on, coronary vascular cells. Whether these enzymes contribute to the formation of adenosine during cardiac hypoxia is unknown.

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Changes in Liver Lipid Composition with Overnight Fasting* (32615)

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We previously observed (1) that fasting for 24 hours resulted in a 34% loss of liver weight, a 19% loss in liver phospholipid, and a significant decrease in the relative proportion (wt. %) of oleate in total liver fatty acids, together with small increases in linoleate and arachidonate. These results indicated a need to study the extent of changes which might result from fasts shorter than 24 hours, such as "overnight," since these intervals have been used in studies of lipid composition (2–4). The following experiment compared the fatty acid composition of liver lipid fractions from rats fasted overnight (ca. 12 hours) with the values from fed rats, as well as with a group fasted for 22 hours.

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Materials and Methods. Male weanling Long-Evans rats were caged individually in suspended, galvanized screen-wire cages. They had been fed a 20% casein–6% cottonseed oil–sucrose diet (5) *ad libitum* for 6 weeks, since this is a period frequently used in nutritional studies with rats fed purified diets. The rats were divided into 4 groups: (a) fed, killed at 8:30 a.m.; (b) fasted from 8 p.m. to 8:30 a.m.; (c) fasted from 8 p.m. to 6 p.m.; (d) fed, killed at 8 p.m., at the start of the fasting period for group b. The average initial weight for all groups ranged from 288 to 296