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The *in vitro* stimulation of lipogenesis and carbohydrate metabolism in liver by potassium ion and diphenylmethylacetyl-N-methylpiperazine

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SUMMARY

An experimental drug, diphenylmethylacetyl-N-methylpiperazine (A-11850), was found to stimulate the incorporation of acetate-1-C^{14}, but not that of mevalonate-2-C^{14}, into the fatty acid and nonsaponifiable fractions of rat liver slices. In addition, the drug increased about equally the oxidation of both glucose-1-C^{14} and glucose-6-C^{14} to $C^{14}O_2$. The incubation of liver slices in a buffer containing a high level of potassium ion stimulated lipogenesis in the same manner as the drug; with potassium ion, however, the oxidation of glucose-1-C^{14} was increased to a much greater extent than that of glucose-6-C^{14}. Potassium ion also stimulated glucose oxidation in liver homogenates, whereas A-11850 did not.

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Potassium ion is known to be involved in the intermediary metabolism of glucose in both plants and animals. The early work on the potassium ion requirements of certain enzymes of glucose metabolism was reviewed by Lehninger (1). Ashmore *et al.* (2) reported a stimulation of glucose metabolism by exogenous potassium ion in rat liver slices, and a similar effect was found in rat liver homogenates (3).

Potassium ion has also been observed to affect lipid metabolism. Potter (4) and Geyer *et al.* (5) demonstrated that addition of potassium ion increases the oxidation of fatty acids by liver cells and slices, and Ashmore *et al.* (6) showed that potassium increases fatty acid synthesis from glucose in liver slices. In addition, Curran and Clute (7) found that cholesterogenesis by liver cell clusters is increased by the addition of potassium. On the other hand, Kline and DeLuca (8) did not find any consistent effect of added potassium ion upon cholesterol and phospholipid synthesis from acetate in liver slices.

During an investigation of the effects of substances upon body lipids by Schmidt *et al.* (9), a group of drugs, which possesses a diarylalkylamine or related structure, was found to lower blood cholesterol levels. When these drugs were tested *in vitro*, however, they were found to enhance both lipogenesis and glucose oxidation. The effects of the drugs were then found to be similar to those of potassium ion in their stimulation of lipogenesis and glucose oxidation. The details of these studies form the subject of this paper. A portion of this work has already been presented (10).

METHODS

Livers were obtained from male Sprague-Dawley rats fed ad lib. a diet of the following composition (expressed as grams per 100 g diet): sucrose (57.0), casein (30), cottonseed oil (5.0), mineral salts (4.0), celluflour (2.0), agar (1.25), choline chloride (0.5), and vitamins (0.19). After decapitation of the rats, the livers were placed in cold saline and then either sliced with a Stadie-Riggs microtome or homogenized. The tissue preparations were then incubated in a Warburg respirometer.

For the investigation of lipogenesis in liver slices, each incubation flask contained 0.2 ml of 10% potassium hydroxide on fluted paper in the center well, 0.2 ml of the radioactive precursor in one sidearm, and 0.3 ml of 3N sulfuric acid in the other sidearm. The liver slices (200 to 250 mg) were added to 3.0 ml of buffer containing the drug or potassium ion. After gassing with 100% oxygen, the precursor was tipped in and incubation proceeded at 37°. After a specified time sulfuric acid was added and 5 minutes later the po-

tassium hydroxide in the center well was removed. The carbon dioxide was later evolved into Hyamine® for counting in a liquid scintillation counter.

The liver slices were hydrolyzed in a mixture of 11 ml of 5% sodium hydroxide in 50% ethanol for 3 hours at 75°. The nonsaponifiable fraction was obtained by extracting the hydrolyzate four times with 10 ml of hexane, and the fatty acids by a similar extraction after acidification of the hydrolyzate with hydrochloric acid. The combined extracts containing the nonsaponifiable fraction were washed once with a dilute solution of sodium acetate; the extracts containing fatty acids were washed once with very dilute acetic acid. Control experiments showed that lipids were usually completely removed from the hydrolyzate by three extractions, and that single washes of the extracts effectively removed contaminating C^{14}-acetate. It should be noted that the proportions of reagents were adjusted so that after 30 seconds of vigorous agitation on a buzzer the emulsions separated cleanly, usually within 1 to 4 minutes. Hexane solutions of the lipids were counted in a liquid scintillation counter.

Two buffers were used for the incubation of slices: a modified Krebs-Ringer phosphate buffer, pH 7.0–7.1, which contained one-half of the standard amount of calcium ion, and a high potassium ion buffer, pH 7.0–7.1, in which all the sodium salts of the preceding buffer were replaced with the corresponding potassium salts. Intermediate concentrations of potassium ion in buffers were obtained by mixing suitable proportions of these two buffers.

Liver homogenates were prepared in a sodium phosphate buffer (3.3 g liver/20 ml buffer) according to the procedure of Wenner *et al.* (3) with a Teflon-glass homogenizer. The homogenates were then incubated in high sodium ion or in high potassium ion buffers which had been reinforced with DPN[1] and cytochrome c (3). In contrast to the experiments with slices, sulfuric acid was not added at the end of the homogenate incubations because it coagulated the tissue and hindered its removal from the incubation flasks. The tissue and the medium were hydrolyzed together, and after acidification of the hydrolyzate, the total lipids were extracted with hexane. Again, the $C^{14}O_2$ was later evolved into Hyamine® for counting.

The use of a bicarbonate buffer would probably have increased lipogenesis above that of the present buffer systems. Drugs at certain levels and conditions, however, can produce a general toxic effect in slices and homogenates which is evidenced by reduced oxygen uptake and carbon dioxide formation. Trapping of carbon dioxide permits the detection of such an effect by an accurate determination of the oxygen uptake. Moreover, the experiments in the present paper were designed primarily to compare controls and treatments, and maximal lipogenesis was considered of secondary importance to a determination of the oxygen uptake.

For the experiments in which the lipids were chromatographed on silicic acid, 1 g of liver slices was incubated in 125-ml Warburg flasks with the acetate-1-C^{14} and other materials increased proportionately. After a 3-hour incubation period, the lipids were extracted from the slices with 10 ml of Delsal's reagent (11) per gram of liver. The extraction was repeated twice and the solvent combined and evaporated *in vacuo*. Lipids were extracted from the residue with several portions of warm hexane and the clear centrifuged solution was added to a 14 mm × 200 mm column of silicic acid (chromatographic silica from the G. Frederick Smith Company). The silicic acid was packed with hexane without any preliminary treatment. Lipid fractions were eluted from the column according to the procedure of Fillerup and Mead (12, 13).

Diphenylmethylacetyl-N-methylpiperazine (A-11850) was prepared by Mr. R. J. Michaels and Mr. H. B. Wright of these laboratories. The acetic acid-1-C^{14} and the C^{14}-glucoses were obtained from the New England Nuclear Company; the purity of the glucoses was confirmed by spraying and determining the radioactivity of a paper chromatogram run in butanol-acetic acid. Unlabeled mevalonic acid and the mevalonic acid-2-C^{14} were obtained from the Isotopes Specialties Company as the diphenylethylenediamine salts and these were regenerated prior to use by ether extraction of an alkaline solution. Acetoacetate was determined by the method of Edson (14) with aniline citrate, the radioactive carbon dioxide being evolved into Hyamine®. Unless otherwise noted, the radiochemical yield of an incubation was defined as the per cent of the added radioactivity recovered in a given substance per 100 mg of fresh tissue per hour. Oxygen uptakes (QO_2) are expressed either as microliter per hour per milligram of fresh tissue slice or as microliter per hour per milliliter of homogenate.

RESULTS

Effect of Graded Levels of A-11850 and Potassium Ion upon Lipogenesis in Rat Liver Slices. The influence of different levels of A-11850 and potassium ion upon the incorporation of acetate-1-C^{14} into lipids is shown in Figure 1, where it can be seen that both

[1] Abbreviations: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; HMG, β-hydroxy-β-methylglutaric acid; CoA, coenzyme A.

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activators produced similar responses. Fatty acid synthesis in the presence of 155 mM potassium ion and 0.65 mM A-11850 was increased to 500% and 700%, respectively, of the control value in Krebs-Ringer buffer. In the same incubations the corresponding data for acetate incorporation into the nonsaponifiable fraction were 250% and 220%. Since the incubations with the drug were carried out in Krebs-Ringer phosphate, each of these incubations was also 5.6 mM in potassium ion. It should also be noted that in the subsequent experiments A-11850 was used at a level of 0.65 mM. To demonstrate stimulation by potassium ion, the previously described buffer was used which contained 155 mM potassium ion. It was not realized until many experiments had been completed that this level of potassium ion was greater than the optimum level. The results of all the incubations using such concentrations of A-11850 and potassium ion are given in Table 1, and the data clearly demonstrate the pronounced stimulation of lipogenesis by both substances. As indicated in Figure 1, the effects of potassium ion upon lipogenesis would probably have been greater at a lower level. In the potassium ion- and drug-stimulated incubations, neither the respiration nor the incorporation of acetate into carbon dioxide and acetoacetate increased above control levels. Moreover, the presence of 100 mM glucose in the medium did not affect the respiration or the acetate incorporation into lipids and carbon dioxide.

Chromatographic Separation of Lipids. Lipid extracts were prepared from incubations (1 g of rat liver slices) which were five times the size of those described in Figure 1. The slices were incubated in either the 155 mM potassium ion buffer or the Krebs-Ringer phosphate buffer containing 0.65 mM A-11850. The respiration and the incorporation of acetate-1-C^{14} into the carbon dioxide and lipids were comparable to those of the smaller incubations. When the lipid extracts were passed over silicic-acid columns, effluent

TABLE 1. Stimulation of Lipogenesis from Acetate-1-C^{14} in Rat Liver Slices*

	Krebs-Ringer Phosphate	A-11850 (0.65 mM)	Potassium Ion (155 mM)
QO_2	1.42 ± 0.05 (9)	1.36 ± 0.04 (7)	1.19 ± 0.02 (5)
	Radiochemical Yield (Per Cent)		
Acetoacetate	1.28 ± 0.04 (2)	1.14 ± 0.10 (2)	1.36 ± 0.04 (2)
Nonsaponifiable lipid	0.03 ± 0.00 (8)	0.11 ± 0.02 (6)	0.08 ± 0.03 (4)
Fatty acids	0.08 ± 0.01 (8)	0.47 ± 0.09 (6)	0.53 ± 0.11 (4)
Carbon dioxide	2.14 ± 0.31 (9)	2.26 ± 0.40 (7)	2.83 ± 0.43 (5)

* Incubations were effected as described in Figure 1. The number of individual livers is given in parentheses and each liver was incubated in triplicate. Each value is expressed as a mean ± the standard error of the mean.

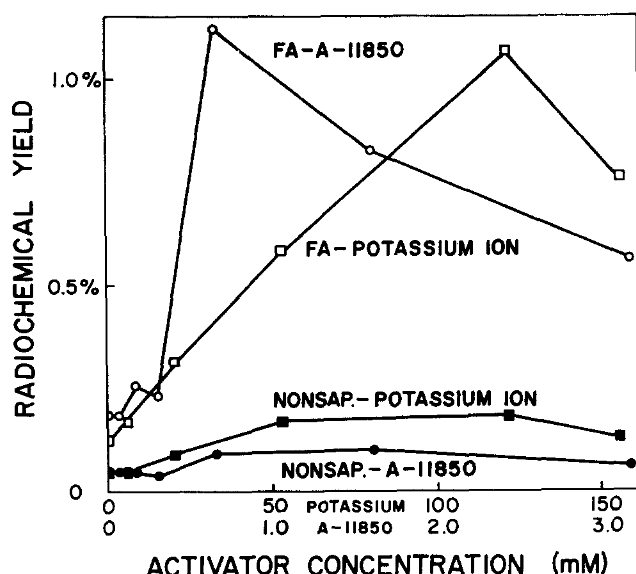

Fig 1. Effect of graded levels of potassium ion and A-11850 upon lipogenesis by rat liver slices. Flask components: 3.0 ml modified Krebs-Ringer phosphate buffer with graded amounts of potassium ion or A-11850 and 200 to 250 mg liver slices in the main well, 0.2 ml 10% KOH in center well, 0.3 ml 3N H_2SO_4 in sidearm, and 0.2 ml of 104 mM acetate-1-C^{14} in other sidearm. Incubated for 3 hours at 37° under 100% oxygen. ○ = A-11850 and fatty acids; ● = A-11850 and nonsaponifiable lipid; □ = potassium ion and fatty acids; ■ = potassium ion and nonsaponifiable lipid.

curves of the radioactivity were obtained as shown in Figure 2. The peaks correspond to those originally described by Fillerup and Mead (12), i.e., hydrocarbons were eluted by hexane, cholesterol esters by 1% ether in hexane, triglycerides by 3% ether in hexane,

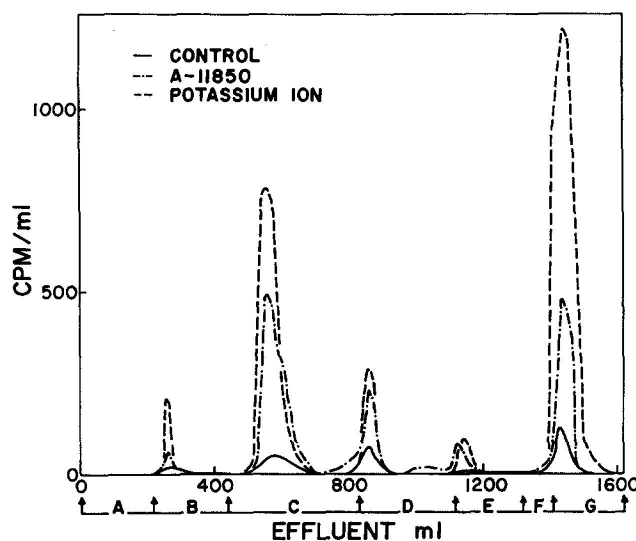

Fig. 2. Chromatography of lipids from liver slice incubations. The incubations were five times the size of those described in Figure 1; silicic-acid column size, 1.4 × 20 cm; fraction size, 24 ml. Solvent mixture *A*, hexane; *B*, 1% diethyl ether in hexane; *C*, 3% ether in hexane; *D*, 10% ether in hexane; *E*, 50% ether in hexane; *F*, 100% ether; *G*, 100% methanol.

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free cholesterol by 10% ether in hexane, fatty acids by 50% ether in hexane, and finally phospholipids by both 100% ether and 100% methanol. However, the free fatty acids in the extracts and the known sample of C^{14}-fatty acids (obtained from a prior incubation) must have become oxidized during their isolation, since in a later report Mead and Fillerup (13) observed that unoxidized fatty acids are eluted directly after the triglycerides. Identification of the substances in each of the other peaks was also confirmed by chromatography with known lipids. Thus the elution patterns shown in Figure 1 demonstrate that acetate-1-C^{14}, under the influence of A-11850 and potassium ion, became incorporated into many types of lipids.

Differential Stimulation of Lipogenesis from Acetate and Mevalonate in Rat Liver Slices. The data obtained when either acetate-1-C^{14} or mevalonate-2-C^{14} was incubated in the presence of 0.65 mM A-11850 with slices from the same liver are shown in Table 2. As may be seen, the expected increase in the labeling of the nonsaponifiable fraction from acetate was obtained, but the labeling from mevalonate remained unchanged. In Table 3 are data from a comparable incubation in the high potassium ion buffer which show a similar differential incorporation into the nonsaponifiable fraction. With neither substance was there any suggestion of an increased labeling from mevalonate in these or other similar experiments.

Stimulation of the Conversion of Glucose to Carbon Dioxide and Lipids in Rat Liver Slices. Because the stimulation of lipogenesis from acetate-1-C^{14} by A-11850 and potassium ion were so similar, it was of interest to see whether this similarity also extended to glucose metabolism. The data in Table 4 show that both substances increased the oxidation of glucose-1-C^{14} and glucose-6-C^{14} to $C^{14}O_2$. It is evident from the data that the oxidation of glucose-1-C^{14} was stimulated to a considerable extent by the addition of potassium ion, while that of glucose-6-C^{14} was almost negligible. On the other hand, A-11850 significantly increased the oxidation of both glucoses, apparently increasing the oxidation of glucose-1-C^{14} slightly more than that of glucose-6-C^{14}. Thus, in contrast to their similar stimulation of lipogenesis from acetate, the effects of A-11850 and potassium ion upon glucose oxidation differ from each other. A similar stimulation of glucose oxidation by A-11850 was also observed when incubations were carried out in a Krebs-Ringer bicarbonate buffer instead of the usual Krebs-Ringer phosphate buffer.

It may be seen in Table 5 that the addition of either A-11850 or potassium ion also increased the incorporation of the C^{14}-glucoses into the nonsaponifiable material and fatty acids. A-11850 increased the incorporation of glucose-6-C^{14} slightly more, on an absolute basis, than that of glucose-1-C^{14}. However, after the addition of potassium ion to liver slices, there was a much greater incorporation into lipids of glucose-6-C^{14} than of glucose-1-C^{14}. It should also be noted that whereas both A-11850 and potassium ion caused larger increases in the amount of lipogenesis from glucose-6-C^{14} than from glucose-1-C^{14}, the converse was observed for the oxidations to $C^{14}O_2$, as is demonstrated in Table 4.

The incorporation of C^{14}-glucose into lipids by liver slices was extremely low even though the oxidation of glucose to $C^{14}O_2$ and lipogenesis from C^{14}-acetate occurred at rates comparable to those observed by other investigators. In fact, of the 10 rats used in Table 4, slices from only 5 livers incorporated enough C^{14}-glucose into fatty acids and the nonsaponifiable fraction to give significant radioactivity.

TABLE 2. Differential Stimulation of Lipogenesis from Acetate and Mevalonate in Liver Slices by A-11850*

Additions	Radiochemical Yield (Per Cent)		
	Nonsaponifiable Lipid	Fatty Acids	Carbon Dioxide
	Precursor: Acetate-1-C^{14}		
None	0.03 ± 0.01	0.02 ± 0.01	0.96 ± 0.03
A-11850	0.20 ± 0.02	0.17 ± 0.01	1.10 ± 0.02
	Precursor: Mevalonate-2-C^{14}		
None	0.90 ± 0.07	0.01 ± 0.01	0.11 ± 0.02
A-11850	0.76 ± 0.01	0.01 ± 0.01	0.09 ± 0.01

* Acetate-1-C^{14}, mevalonate-2-C^{14}, and A-11850 were at 0.65 mM concentrations. Liver slices were incubated in the modified Krebs-Ringer phosphate for 3 hours at 37° under 100% oxygen. Recoveries are expressed as radiochemical yields and each value is the mean of triplicate incubations. All the data were obtained with one rat liver.

TABLE 3. Differential Stimulation of Lipogenesis from Acetate and Mevalonate in Liver Slices by Potassium Ion*

Medium	Radiochemical Yield (Per Cent)		
	Nonsaponifiable Lipid	Fatty Acids	Carbon Dioxide
	Precursor: Acetate-1-C^{14}		
KRP-buffer	0.04 ± 0.00	0.13 ± 0.00	1.83 ± 0.17
Potassium ion buffer (155 mM)	0.16 ± 0.00	0.75 ± 0.16	1.99 ± 0.00
	Precursor: Mevalonate-2-C^{14}		
KRP-buffer	1.09 ± 0.04	0.03 ± 0.00	0.14 ± 0.03
Potassium ion buffer (155 mM)	1.04 ± 0.09	0.04 ± 0.01	0.14 ± 0.02

* Incubations were carried out as described for those in Table 2.

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TABLE 4. STIMULATION OF GLUCOSE OXIDATION IN LIVER SLICES*

Rat	Radiochemical Yield of CO_2 (Per Cent)	
	G-1-C14	G-6-C14
	Krebs-Ringer Phosphate	
3	0.32 ± 0.02	0.10 ± 0.01
4	0.17 ± 0.01	0.05 ± 0.01
5	0.23 ± 0.02	0.06 ± 0.00
6	0.20 ± 0.02	0.07 ± 0.01
7	0.09 ± 0.01	0.08 ± 0.01
8	0.19 ± 0.01	0.08 ± 0.00
9	0.19 ± 0.00	0.08 ± 0.00
10	0.18 ± 0.01	0.07 ± 0.00
11	0.24 ± 0.01	0.09 ± 0.01
12	0.19 ± 0.01	0.07 ± 0.00
	A-11850 (0.65 mM)	
3	0.56 ± 0.02 (0.24)	0.23 ± 0.04 (0.13)
4	0.32 ± 0.05 (0.15)	0.12 ± 0.01 (0.07)
6	0.41 ± 0.02 (0.21)	0.15 ± 0.01 (0.08)
10	0.40 ± 0.01 (0.22)	0.12 ± 0.02 (0.05)
11	0.37 ± 0.02 (0.13)	0.23 ± 0.02 (0.14)
12	0.32 ± 0.00 (0.13)	0.15 ± 0.01 (0.08)
	($\bar{X}$ = 0.18 ± 0.02)	($\bar{X}$ = 0.09 ± 0.02)
	High Potassium Ion Buffer (155 mM)	
3	1.23 ± 0.26 (0.91)	0.14 ± 0.01 (0.04)
5	0.86 ± 0.04 (0.63)	0.13 ± 0.01 (0.07)
7	0.29 ± 0.03 (0.20)	0.13 ± 0.00 (0.05)
8	0.36 ± 0.04 (0.17)	0.09 ± 0.00 (0.01)
9	0.33 ± 0.04 (0.14)	0.08 ± 0.01 (0.00)
	($\bar{X}$ = 0.41 ± 0.17)	($\bar{X}$ = 0.03 ± 0.01)

* Glucose was added to the medium at a level of 0.4 mM. The incubations proceeded for 90 minutes at 37° under 100% oxygen. Each yield is a mean of triplicate incubations and is expressed as a mean ± standard error of the mean. Values in parentheses are the increases in glucose oxidation after the addition of either A-11850 or potassium ion.

Actions of A-11850 and Potassium Ion upon Acetate and Glucose Metabolism of Rat Liver Homogenates. In order to determine whether A-11850 and potassium ion would stimulate metabolism in a cell-free system, experiments using homogenates were compared with those of slices (Table 6). As reported earlier by Wenner *et al.* (3), the addition of potassium ion was found to increase both the respiration and glucose oxidation of liver homogenates reinforced with cytochrome c and DPN. On the other hand, 0.65 mM A-11850 depressed respiration but not glucose oxidation. In two separate experiments the oxidation of acetate-1-C^{14} to $C^{14}O_2$ was also found to be increased by potassium ion but not by A-11850; lipogenesis was slightly inhibited in both experiments. These actions are in contrast to those observed in slices where both A-11850 and potassium ion caused stimulations.

TABLE 5. STIMULATION OF C^{14}-GLUCOSE CONVERSION TO LIPIDS IN LIVER SLICES

Rat		Radiochemical Yield (Per Cent)*	
		Glucose-1-C14	Glucose-6-C14
6	**Krebs-Ringer Phosphate**		
	Nonsaponifiable lipid	0.19 ± 0.03	0.42 ± 0.04
	Fatty acids	0.74 ± 0.15	1.3 ± 0.1
	A-11850 (0.65 mM)		
	Nonsaponifiable lipid	1.4 ± 0.2 (1.2)	3.1 ± 0.4 (2.7)
	Fatty acids	7.6 ± 0.9 (6.9)	15.0 ± 1.3 (13.7)
12	**Krebs-Ringer Phosphate**		
	Nonsaponifiable lipid	0.19 ± 0.05	0.41 ± 0.05
	Fatty acids	0.56 ± 0.06	1.1 ± 0.1
	A-11850 (0.65 mM)		
	Nonsaponifiable lipid	0.73 ± 0.06 (0.5)	1.9 ± 0.5 (1.5)
	Fatty acids	3.1 ± 0.2 (2.5)	7.4 ± 1.3 (6.3)
7	**Krebs-Ringer Phosphate**		
	Nonsaponifiable lipid	0.00 ± 0.03	0.12 ± 0.03
	Fatty acids	0.19 ± 0.03	0.67 ± 0.05
	High Potassium Ion Buffer (155 mM)		
	Nonsaponifiable lipid	0.00 ± 0.04 (0.00)	0.72 ± 0.06 (0.6)
	Fatty acids	0.33 ± 0.01 (0.14)	2.8 ± 0.6 (2.1)
9	**Krebs-Ringer Phosphate**		
	Fatty acids	0.26 ± 0.05	0.52 ± 0.09
	High Potassium Ion Buffer (155 mM)		
	Fatty acids	0.32 ± 0.02 (0.04)	0.77 ± 0.08 (0.25)

* Actual yields × 10^3. Triplicate incubations were carried out as described in Table 4. Values in parentheses are the increases in lipogenesis after the addition of either A-11850 or potassium ion.

TABLE 6. STIMULATION OF GLUCOSE OXIDATION IN LIVER HOMOGENATES*

Oxygen Uptake ($\mu l/hr/ml$)	Radiochemical Yield of Carbon Dioxide (Per Cent)	
	Glucose-1-C14	Glucose-6-C14
High Sodium Ion Buffer		
221 ± 14	0.30 ± 0.00	0.008 ± 0.001
A-11850 (0.65 mM) in Sodium Ion Buffer		
143 ± 5	0.31 ± 0.02	0.007 ± 0.000
Potassium Ion Buffer (88 mM)		
284 ± 11	0.34 ± 0.01	0.029 ± 0.001

* The homogenates were incubated in air for 1 hour at 37°. C^{14}-glucose was present at 10 mM. The $C^{14}O_2$ yields and the oxygen uptakes are expressed as the means ± the standard error of the mean for duplicate and quadruplicate incubations, respectively.

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DISCUSSION

The dependence of lipogenesis upon glucose metabolism has been investigated and reviewed by Siperstein and Fagan (15). They presented evidence that in liver homogenates the effects of glucose metabolism are mediated through the formation of reduced pyridine nucleotides. In addition, Ashmore *et al.* (6) have shown that both glucose metabolism and fatty acid synthesis are elevated by potassium ion. The conclusion is that lipogenesis can be regulated by glucose catabolism and that this in turn may be influenced by potassium ion. In the *in vitro* systems used in this paper, which are similar to those used by many other investigators, a synthetic drug as well as potassium ion exerted such a role. The increases in lipogenesis and oxidation of glucose in the presence of either A-11850 or potassium ion are further examples of an increased glucose oxidation being accompanied by an increased lipogenesis; glucose oxidation and lipogenesis are known to be increased by the addition of DPN and TPN to liver homogenates (15) and also by the addition of insulin to adipose tissue (16).

The data in Tables 2 and 3 demonstrate that in liver slices both the drug and potassium ion increase labeling of the nonsaponifiable fraction from acetate-1-C^{14} but not from mevalonate-2-C^{14}. Furthermore, as shown in Table 1, the radioactivity of acetoacetate in the medium after incubation with labeled acetate was found to be the same in both control and stimulated incubations. (In two separate experiments the absolute amounts of acetoacetate in the medium were also found to be the same.) In contrast to the earlier hypothesis that acetoacetic acid is formed by deacylation of acetoacetyl-CoA, Lynen *et al.* (17) reported that liver acetoacetate is derived mainly from the cleavage of HMG-CoA, and therefore its specific activity should reflect that of HMG-CoA. A consideration of the above observations suggests that A-11850 and potassium ion do not affect the specific activity of HMG-CoA, but that they stimulate some process(es) in the conversion of HMG-CoA to mevalonate. The evidence presented here, together with that of other investigators, suggests that this stimulatory effect upon the conversion is mediated through an increased oxidation of glucose and its concomitant formation of reduced TPN which is known to be the coenzyme involved in the conversion of HMG-CoA to mevalonic acid (18).

Although the actions of A-11850 and potassium ion upon lipogenesis from acetate-1-C^{14} and mevalonate-2-C^{14} were found to be very similar, differences were observed in their stimulation of glucose metabolism. Examination of the data in Tables 4 and 5 shows that although each of the substances increased the conversion of glucose-1-C^{14} and glucose-6-C^{14} to carbon dioxide and lipids, potassium caused a proportionately greater increase in the oxidation of glucose-1-C^{14} than of glucose-6-C^{14}, and also a proportionately greater stimulation of lipogenesis from glucose-6-C^{14} than from glucose-1-C^{14}. These data suggest that there are changes in the relative quantities of glucose passing through Embden-Meyerhoff glycolysis and the hexose phosphate shunt. However, in view of the discussion by Katz and Wood (19), any quantification based only upon glucose-1-C^{14} and glucose-6-C^{14} would be equivocal.

Potassium ion will not increase glucose oxidation in some types of homogenates; no activation was observed in two trials with homogenates prepared according to Dituri *et al.* (20) with added nicotinamide. However, when plain liver homogenates were reinforced with cytochrome c and DPN, an increased glucose oxidation was found in the presence of potassium ion (Table 6), as Wenner *et al.* (3) reported earlier. The elevated oxidation of acetate after addition of potassium ion to the homogenate may be due to its effect upon the acetate activating enzyme as Von Korff (21) observed in heart extracts.

Although both substances increased metabolism in a cellular system (liver slices), only potassium ion stimulated metabolism in liver homogenates. It may be that A-11850 is involved with intracellular potassium ion, although we have no evidence for it; A-11850, however, is structurally related to many antihistaminic drugs, and Judah (22) has reported that such drugs enable liver slices to accumulate potassium ion. It is also of interest that antihistaminics are known to lower serum potassium ion levels in rats (23) and to produce hypoglycemia in man and animals (24, 25). In this connection it should be noted that Hastings *et al.* (26) discussed the possibility of such a cellular relationship between insulin and potassium ion, and that insulin has been found to increase the potassium content of rat diaphragm during incubation *in vitro* (27) and to reduce hyperkalemia (28). These observations emphasize certain relationships among insulin, potassium ion, and drugs having antihistaminic activity.

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