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THE FORMATION OF FREE AND LIPID MYO-INOSITOL IN THE INTACT RAT

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SUMMARY

1. The incorporation of ^{14}C into the free and lipid-bound myo-inositol of rat brain, liver, kidney and blood was measured after the injection of [$^{14}C_6$]glucose and other radioactive substances into young and adult rats.

2. In brain, radioactivity appeared in free inositol and in lipid–inositol bound in phosphatides and phosphatidopeptides after the injection of [$^{14}C_6$]glucose. Highest specific activity was observed in free blood inositol. The time curves of incorporation are consistent with the involvement of free inositol in the assembly of inositol phosphatide in brain; however, the inositol-containing lipids in phosphatidopeptides appear to belong to a different pool.

3. Both free and phosphatide inositol of liver and kidney also became labeled, with higher activity occurring in the free inositol. Specific activities in young rats were about twice as great as in adult ones. Sodium [2-^{14}C]pyruvate gave rise to labeled inositol (both free and bound) in brain, liver and kidney, but no incorporation was obtained with sodium [1-^{14}C]acetate or sodium [6-^{14}C]glucuronate.

4. In addition to these findings data on the amounts of inositol isolated and on the incorporation of ^{14}C of glucose and pyruvate into phosphatide glycerol are presented.

INTRODUCTION

Inositol occurs in the free form in considerable amounts in all tissues of the rat that have been studied[1–4] and has also been shown to be a component part of certain phospholipids, the phosphoinositides[5].

Much recent work has served to clarify the chemical structure of the phosphoinositides[6–12]. These complex lipids, in addition to occurring in a form easily extracted by lipid solvents, also are closely linked to several protein fractions in brain[13]. The assembly of the phosphatidylinositol molecule in both liver and brain has been elucidated[14–18], and observations on the metabolism of the more highly phosphorylated inositides have been reported[13,19–28]. Although there have been suggestions that the phosphoinositides may be involved in some transport phenomena across membranes[29], their exact function is at present unclear.

Both the formation and the function of free inositol and its relation to glucose,

which in brain is essentially the sole source of energy under normal circumstances, on the one hand, and to the inositol lipids on the other are as yet unexplored. That the mammalian organism can form inositol from glucose has been shown by HALLIDAY AND ANDERSON[30], DAUGHADAY, LARNER AND HARTNETT[31] and in a recent convincing study by FREINKEL AND DAWSON[32].

This communication deals with the incorporation of radioactive carbon into the free and bound inositol of brain and other organs. Some information about the radioactivity of phosphatide glycerol and of free blood and tissue glucose is also reported. A part of this study has been presented previously[33].

METHODS

Experimental procedure

Sprague–Dawley rats, obtained from the Charles River Laboratories, Wilmington, Mass., were injected intraperitoneally with approx. 0.1 μC of radioactive material per gram of body weight and decapitated after suitable time periods. Blood was collected at the time of decapitation in 10% trichloroacetic acid or, in early experiments, in chloroform–methanol (2:1, v/v). Wherever trichloroacetic acid was used, it was removed from the supernatant solution by exhaustive extraction with ether.

The entire brain was rapidly removed from the head and blotted with pressure between pieces of hard filter paper (Schleicher and Schüll No. 576) to remove as many of the blood vessels and meninges as possible. Any visible vessels which remained were teased away with a pair of forceps. The brain was quickly weighed and homogenized in 19 vol. of chloroform–methanol (2:1, v/v) in either a Waring blendor or a Virtis homogenizer.

Whenever kidneys or livers were to be examined, these organs were removed from the peritoneal cavity, thoroughly rinsed in isotonic saline, blotted, weighed and homogenized in chloroform–methanol.

Between 2 and 7 animals were used in each experiment and their organs pooled.

Isolation of tissue fractions

After filtration of the homogenate and rinsing of the lipid-free tissue residue with further portions of solvent mixture, an aqueous extract containing the water-soluble constituents of the tissue and an acid chloroform–methanol extract containing the phosphatidopeptides were prepared as previously described[13].

The total lipid extract was washed six times with KCl-containing water and theoretical upper phase according to the method of FOLCH, LEES AND SLOANE STANLEY[34] and taken to dryness on a rotary evaporator. The dry lipid film was rinsed several times with dry acetone to remove most of the cholesterol before being dissolved in a small volume of dry chloroform–methanol (2:1, v/v) for transfer to a Florisil (Floridin Company, Tallahassee, Fla.) column[35].

3 g of Florisil per 100 mg lipid were used. Two fractions were collected, one with dry chloroform–methanol (galactolipids) and the second with chloroform–methanol (2:1, v/v) saturated with water (about 7%) (phosphatides). Incorporation of radioactivity into galactolipids will be reported elsewhere.

Hydrolysis of inositol-containing lipids

The fractions containing bound inositol (phosphatides and phosphatidopeptides)

were concentrated to a small volume, transferred to hydrolysis tubes and dried. Hydrolysis was carried out in 1–2 ml of 6 N HCl at 109° for 40 h in sealed tubes. After cooling the hydrolysate was extracted repeatedly with ether, filtered through paper into a small evaporating dish and dried *in vacuo* over NaOH. The dry residue was taken up in water for the isolation of inositol and, where indicated, of glycerol.

Isolation of inositol, glucose and glycerol

Aqueous tissue extracts and water-soluble hydrolysis products were deionized by passage through Amberlite MB-1 columns. Glucose and inositol in the concentrated effluents of the aqueous extracts were separated on sheets of Whatman No. 1 or 3MM paper. The bands of carbohydrate were cut out and eluted with water. Carrier was added after analysis.

To the ether-extracted, deionized, dried and analyzed phospholipid hydrolysates carrier inositol and glycerol to give a total of about 15 mg of each were added. Only carrier inositol was added to the phosphatidopeptide hydrolysates. The solutions were concentrated to about 0.5–1.0 ml and 7 ml of absolute ethanol were added. Inositol was allowed to crystallize at —10°, centrifuged and the precipitate washed twice with ethanol. The supernatant fluid and the ethanol washings were combined, evaporated and tribenzoin prepared[36]. Both the glycerol derivative and the inositol were recrystallized to constant specific activity.

Analytical methods

Glucose was analyzed with glucose oxidase (Glucostat, Worthington Chemical Corp., Freehold, N.J.), adapted to a range of 2–15 μg in a total volume of 2.0 ml or with the orcinol method of SØRENSEN AND HAUGAARD[37] adapted to a range of 1–7 μg[38]. Inositol was determined by the method of LEBARON, FOLCH AND ROTHLEDER[39], after removal of glycerol where necessary as described by these authors. Glycerol was estimated by a micromethod, based on the use of periodic acid. To samples containing 1–6 μg of glycerol in 200 μl, 100 μl of 0.002 M periodic acid freshly diluted from a 0.1 M stock solution were added. After exactly 10 min the reaction was stopped by the addition of 3.0 ml of water. The difference in absorbancy at 222 mμ between the water blank and the sample represents the disappearance of periodic acid and is proportional to the amount of glycerol present.

Since most of the glycerol determinations were carried out on deionized hydrolysates of inositol-containing lipid, it was important to establish that inositol did not react with periodic acid under the conditions used. No decrease in absorbancy was observed if an amount of inositol equal to the glycerol present was added to the samples.

Labeled substances and determination of radioactivity

Radioactive compounds were obtained from the New England Nuclear Corp., Boston, Mass. or the California Corporation for Biochemical Research, Los Angeles, Cal.

Radioactivity measurements were made with a windowless gas-flow counter[40] or, for low activities, with a counting system including a low-background automatic sample changer (Nuclear Chicago Corp., Model C115). To make the two types of measurements comparable, a factor correcting for the different geometry was used. Glucose was plated as its phenylosazone; glycerol was counted as tribenzoin[36]. Inositol

was plated directly from ethanol. All counting was done on stainless-steel planchets with a plating area of 1.6 cm^2, and suitable self-absorption factors were employed[41].

Chromatographic separation of carbohydrates

Paper chromatograms were developed by the descending technique with acetone–water (9:1, v/v) (3 h) or propan-2-ol–acetic acid–water (3:1:1, v/v) (15 h). Carbohydrate markers were located by the method of TREVELYAN, PROCTER AND HARRISON[42] or by spraying with aniline oxalate reagent[43].

RESULTS

Isolation of free inositol

In the procedure employed for the isolation of tissue components small molecules which occur in the free state will be partly extracted into the initial lipid extract with chloroform–methanol. The remainder will be removed from the tissue by subsequent treatment of the lipid-free residue with water. The amounts of free inositol shown in column 1 of Table I represent only this latter fraction. If the lipid extract is washed according to FOLCH, LEES AND SLOANE STANLEY[34] and the washings concentrated, dialyzed and deionized, the dialysate contains additional amounts of water-soluble components. Such dialysates obtained from brain contained approx. 30 % of the sum of the inositol in the two fractions (washings of lipids plus water extract of lipid-free tissue residue).

TABLE I

AMOUNTS OF INOSITOL AND GLYCEROL ISOLATED FROM RAT TISSUES

Results are expressed as μg per g fresh weight ± S.D. The number of isolations is indicated in parentheses.

	Free inositol	*Phosphatide inositol*	*Phosphatide glycerol*
Brain	730 ± 104 (12)	239 ± 54 (10)	1850 ± 527 (10)
Liver	505 ± 327 (5)	490 ± 140 (5)	1475 ± 305 (5)
Kidney	879 ± 223 (7)	252 ± 47 (5)	836 ± 197 (5)

Only very small amounts of free inositol on the order of 20 μg/ml were isolated from rat blood, in reasonable agreement with the finding of 30 μg/ml by DAUGHADAY AND LARNER[44].

Isolation of inositol and glycerol from phosphatides

Table I also indicates the amounts of phosphatide inositol and glycerol obtained after hydrolysis of the chloroform–methanol–water eluate from Florisil. Twice as much lipid inositol was found in liver as in brain or kidney. The quantity of inositol found in the phosphatidopeptide fraction after isolation was about 20 μg/g fresh brain.

Phosphatide glycerol was highest in brain, somewhat less in liver and about half as great in kidney. It must be emphasized that these values do not represent the quantities actually present. From published data[45] it can be calculated that in brain and liver about 4 mg of glycerol per gram fresh tissue and in kidney about 2.7 mg per gram fresh tissue occur in phosphatides. Thus between one-third and one-half of the expected amounts were finally obtained.

Specific activities of free blood and brain glucose

Since the original purpose of this investigation was the study of the biosynthesis of inositol and other constituents of brain, it was of interest to determine the changes in specific activity of blood and brain glucose. Fig. 1 shows the initial rapid decrease of blood glucose specific activity followed by a leveling off after 4 h at a value which was less than 4% of the activity at 0.5 h.

Brain glucose rapidly decreases in amount after death. Thus only small quantities of free glucose were found in this organ. This glucose is presumed to be true brain glucose since care was taken to remove as many of the blood vessels as possible before the brain was subjected to extraction with chloroform–methanol. The specific activity of brain glucose reached its peak at about 0.5 h after injection and decreased at a much slower rate than blood glucose.

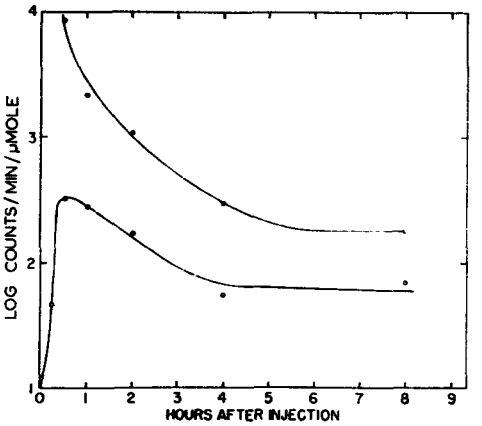

Fig. 1. Time curves of the specific activity of blood (●—●) and brain (○—○) glucose in young rats. Animals were injected intraperitoneally with 0.1 μC of [$^{14}C_6$]glucose per gram of body weight.

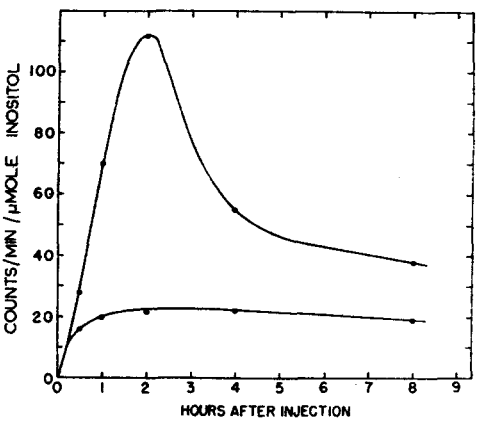

Fig. 2. Time course of labeling of the free inositol of blood (●—●) and brain (○—○) after the injection of [$^{14}C_6$]glucose intraperitoneally into young rats. 0.1 μC per gram of body weight was administered.

With liver extracts some difficulty was experienced in separating free glucose and inositol on paper. Acetone–water (10:3, v/v) rather than (9:1, v/v) as the developing solvent gave the best separation. A large discrete spot appeared between glucose and inositol in all liver extracts and is undoubtedly one of the oligosaccharides which have been reported[46]. On hydrolysis only glucose was obtained and in one experiment this glucose was isolated and counted. It had a specific activity of 18.6 counts/min/μmole compared to a free liver glucose specific activity of 484 counts/min/μmole.

Time course of incorporation of glucose carbon into inositol and phosphatide glycerol

After the injection of labeled glucose into young rats radioactivity rapidly appears in the free tissue inositol. The time course of this incorporation into the inositol of blood and brain is shown in Fig. 2. After 0.5 h the specific activity in brain had almost reached its maximum and leveled off, whereas it continued to rise at a virtually constant rate for at least 1.5 h in blood. While brain inositol maintained its specific activity at a constant level between 1 and 8 h after injection, the activity

in blood had dropped to about one-third of its maximum by this time although it was still twice as great as in brain. The total activity incorporated into free brain inositol was approx. 0.01 % of the injected dose.

Fig. 3 shows similar time curves for inositol bound in brain lipids extracted with neutral and acid solvents. Although phosphatide inositol reached the same specific activity as free inositol at 3 h after injection, and maintained it at this level until at least 8 h, the initial rate of incorporation was much slower. Slower still was the labeling of phosphatidopeptide inositol which reached and maintained a level of activity one quarter as high as phosphatide inositol.

The specific activity time curve of glycerol isolated from the brain phosphatide fractions is given in Fig. 4. It should be stressed that the individual phosphatides were not separated and that this curve represents the activity of the total glycerol esterified as phosphatide. It is conceivable that the glycerol moiety of inositol lipid might exhibit a different pattern (see DISCUSSION). A leveling off of the specific activity after 2 h similar to that seen in the inositol fractions was found.

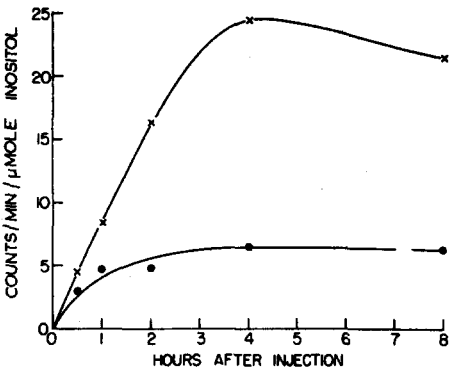

Fig. 3. Time course of labeling of lipid inositol in brain. Phosphatide inositol (×—×), phosphatidopeptide inositol (●—●). Injections as in Fig. 2.

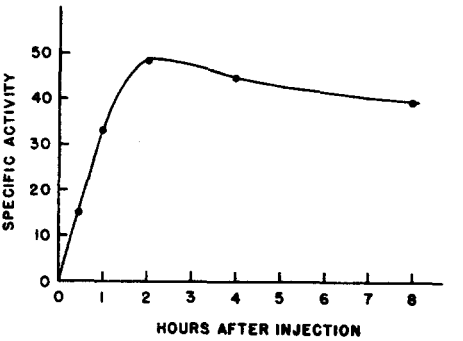

Fig. 4. The incorporation of radioactivity from [$^{14}C_6$]glucose into brain phosphatide glycerol as a function of time. Injections as in Fig. 2. Specific activity is expressed as counts/min per μmole.

Radioactivity in inositol of different tissues after the injection of different labeled substances

Since liver and kidney are both metabolically active and rich in free inositol, it was of interest to compare the incorporation of radioactive glucose carbon into the free inositol of these two organs with the incorporation into brain and blood inositol. A period of 90 min was allowed to elapse between injection and sacrifice of the animals. The middle column of Table II indicates higher specific activities of inositol in liver and kidney than in brain in young rats. The very small amounts of inositol in blood were labeled to a substantially greater extent.

If these data are compared with analogous values obtained from adult rats, the blood inositol specific activity is seen to be markedly lower. Although a decrease was observed in the other organs as well, brain still showed the lowest activity.

In animals injected with radioactive pyruvate the free inositol of the three organs examined was also labeled. The high activity of liver inositol is noteworthy. These data have been normalized to a specific activity of 2000 counts/min/μmole of blood

glucose which was approximately the level reached at 1.5 h after the injection of 0.1 μC of [$^{14}C_6$]glucose per gram of body weight.

Inositol is known to be catabolized to glucuronic acid by the kidney[47] and C_2 units may be involved in its synthesis from glucose by yeast[48]. Sodium [6-^{14}C]-glucuronate and sodium [1-^{14}C]acetate as well as sodium [2-^{14}C]pyruvate and [$^{14}C_6$]-glucose were injected into different groups of 18–20-day-old animals and the free tissue inositol isolated and assayed for radioactivity. Data in Table III are calculated on the basis of injected radioactivity rather than blood glucose activity in order to place them on a uniform basis. On this basis brain and kidney inositol were labeled only 10% and 20% as much from pyruvate as from glucose while both precursors were about equally effective in liver. In none of the three organs could appreciable radioactivity be detected in inositol after the injection of either acetate or glucuronate. The latter did not cause the lipids to become labeled either.

TABLE II

RADIOACTIVITY OF FREE TISSUE INOSITOL

Animals were injected with 0.1 μC of [$^{14}C_6$]glucose (G) or sodium [2-^{14}C]pyruvate (P) per gram of body weight and sacrificed after 1.5 h. The results are expressed as counts/min/μmole of inositol. Specific activities are normalized to a blood glucose specific activity of 2000 counts/min/μmole. Young animals were 20–21 days old. The number of experiments is indicated in parentheses.

	Adult rats (G)	*Young rats (G)*	*Young rats (P)*
Blood	39.7 ± 14.5 (6)	119.7 ± 17.4 (3)	—
Liver	17.5	42.2 ± 9.4 (2)	86.2
Kidney	24.1	32.5 ± 9.4 (3)	30.5
Brain	12.4 ± 3.8 (7)	21.9 ± 5.8 (4)	11.2

TABLE III

RADIOACTIVITY OF FREE TISSUE INOSITOL AFTER THE INJECTION OF DIFFERENT SUBSTANCES

Animals varied in age between 17 and 21 days and were sacrificed 1.5 h after injection. The results are expressed as counts/min/μmole of inositol, corrected to 0.1 μC injected per gram of body weight.

	[$^{14}C_6$]Glucose	*Sodium [2-^{14}C]pyruvate*	*Sodium [6-^{14}C]glucuronate*	*Sodium [1-^{14}C]acetate*
Brain	18.4 (3)	2.2	0	1.2
Liver	26.7 (3)	17.0	0.8	0
Kidney	30.4 (3)	6.0	0	1.9

Radioactivity in phosphatide constituents

When the inositol combined in phosphatides was isolated and its radioactivity determined, the data in Table IV were obtained. In both the young and adult animals liver inositol had the lowest specific activity, brain inositol the highest. It should be pointed out that in brain the phosphatide inositol activity was much closer to that of free inositol than in the other organs. Activities in all organs were about twice as great in young animals as in mature ones. After pyruvate injection all three organs gave identical specific activities in the lipid inositol fraction.

The total phosphatide glycerol on the other hand had the highest specific activity in brain when pyruvate was injected into young animals, but in liver when glucose was the substance administered to either young or adult rats (Table V). The same difference due to the age of the animals was observed with phosphatide glycerol as with either free or bound inositol (Tables II and IV). In one experiment with weanling rats small amounts of free glycerol were obtained from the washings of the lipids of liver and brain. This glycerol had a specific activity (corrected to 2000 counts/min per μmole of blood glucose) of 93.8 counts/min/μmole in brain and 175 counts/min per μmole in liver, *i.e.* about twice as high as the esterified glycerol in these tissues.

TABLE IV

RADIOACTIVITY IN PHOSPHATIDE INOSITOL

Animals were injected with 0.1 μC of [$^{14}C_6$]glucose (G) or sodium [2-^{14}C]pyruvate (P) per gram of body weight and sacrificed after 1.5 h. The results are expressed as counts/min/μmole of inositol. Specific activities are normalized to a blood glucose specific activity of 2000 counts/min/μmole. Young animals were 20–21 days old. The number of experiments is indicated in parentheses.

	Adult rats G	*Young rats* G	*Young rats* P
Brain	6.4 (2)	15.6 (3)	7.3
Liver	2.8	5.6 (2)	7.4
Kidney	5.5	9.4 (2)	7.4

TABLE V

RADIOACTIVITY IN PHOSPHATIDE GLYCEROL

Animals were injected with 0.1 μC of [$^{14}C_6$]glucose (G) or sodium [2-^{14}C]pyruvate (P) per gram of body weight and sacrificed after 1.5 h. The results are expressed as counts/min/μmole of glycerol. Specific activities are normalized to a blood glucose specific activity of 2000 counts/min/μmole. Young animals were 20–21 days old. The number of experiments is indicated in parentheses.

	Adult rats G	*Young rats* G	*Young rats* P
Brain	22.2 (2)	44.5 (2)	48.7
Liver	40.0	109.6 (2)	39.1
Kidney	32.2	42.2 (2)	22.4

DISCUSSION

Although a number of authors[1–4] have reported the concentration of free and bound inositol in the brain, liver and kidney of the rat, the agreement among the values found is not very good. This may be the result of differences in the preparation of samples for analysis which might give rise to losses, of the method of assay (chemical or microbiological) or of the strain of animals used. The data reported in this paper, which are claimed to represent minimum rather than total amounts because of losses in the total lipid extract, are in agreement with the conclusion that brain and kidney contain higher amounts of free inositol than liver, but that liver yields larger quantities of phosphatide inositol than either of the other two tissues. If allowance is made

for the fact that about one-third of the free brain inositol remained in the lipid extract, the values for isolated free inositol agree quite well with those of BATTAGLIA *et al.*[3] but are for unexplained reasons appreciably higher than those reported by DAWSON AND FREINKEL[4].

Considerable work has been done to elucidate the chemistry of the inositol-containing lipids since FOLCH demonstrated that inositol metadiphosphate can be isolated from beef brain cephalin[49]. It is now clear that mono-, di- and triphosphoinositides occur in brain[6,8,10–12], although the more highly phosphorylated inositides have been shown to exist in other tissues only in very small quantities[11]. These are partly found associated with proteins (proteolipids, viscous protein, phosphatidopeptides) in brain as shown in a recent communication from this laboratory[13] whereas monophosphoinositide appears in the proteolipid-free lipid extract together with di- and perhaps triphosphoinositide.

Triphosphoinositide seems to be synthesized from monophosphoinositide *via* the diphospho-compound as shown by incorporation studies with ^{32}P (see refs. 10, 25, 26 and 28). The assembly of the monophosphoinositide molecule, as elucidated by the work of AGRANOFF, BRADLEY AND BRADY[14] and PAULUS AND KENNEDY[16,17] with kidney and liver enzyme preparations respectively, results from the reaction of free inositol with cytidinediphosphate diglyceride. This reaction also occurs in brain preparations as demonstrated by THOMPSON *et al.*[18].

Thus it would appear that the free inositol pool of the tissue is involved with the replenishment of the phosphoinositides. Our experiments show that the 90-min specific activities are lower for phosphoinositide inositol than for free inositol in all tissues studied in both young and adult animals (Tables II and IV), consistent with a precursor–product relationship. However, within 3 h the inositol moiety of the brain phosphoinositides soluble in neutral solvents reaches the same specific activity as free inositol in brain in contrast to the difference at earlier time periods (Fig. 1). On the other hand the phosphoinositides linked in phosphatidopeptides which incorporate ^{32}P at seemingly the same rapid rate as those in the lipid extract[13] assimilate glucose carbon into inositol at a much slower rate. This is consistent with the conclusion of AGRANOFF[50] derived from labeling studies with [^{3}H]inositol that different routes of incorporation of inositol into lipids and into phosphatidopeptides exist and with our previous suggestion[13] that the renewal rates of the constituent parts of these molecules might differ from each other. However, in rat liver FRIEDEL AND HERKEN[51] detected no difference in the rate of incorporation of either ^{32}P- or ^{14}C-labeled inositol into the lipid-soluble and protein-bound inositol phosphatides.

It appears that the organs of the young rat are better able to convert glucose to inositol than those of the mature animal. The specific activities of both free and phosphatide inositol of the three organs investigated are lower in adult than in young animals 90 min after injection. The decrease is about equal for the two inositol fractions (young: adult equals about 2) so that the specific-activity ratio of free to lipid inositol is not greatly different at the two ages, although kidney and liver showed roughly two and four times as high a ratio as brain. In the case of brain therefore, no blood–brain barrier effect need be invoked to explain the lower adult radioactivities with ^{32}P and glucose, since they may reflect decreased metabolic activity in all organs as suggested by DOBBING[52]. The relatively high specific activities of free blood inositol would make it appear that none of the tissues studied is the primary

site of the conversion of glucose to inositol. Although the ability of the mammalian organism to carry out this conversion has been established[20-22], virtually no information is presently available as to its pathway. It is likely that it is not a simple one and involves the breakdown of the carbon chain of glucose as suggested by LOEWUS AND KELLY for parsley leaves[53] and by CHARALAMPOUS for yeast[48]. The latter author, on the basis of the differential incorporation of C-1, C-2 and C-6 of glucose and the ability of sodium acetate and formate to function as inositol precursors, envisaged a possible condensation of a C_4 with a C_2 fragment. Our failure to find radioactivity in inositol after the injection of acetate would tend to exclude this compound as a direct precursor, but the incorporation from pyruvate, especially in liver, is intriguing. While pyruvate presumably does not penetrate into the brain, so that its observed metabolism in this organ is a reflection of its previous conversion to glucose in the liver[54], the relatively high specific activity of free inositol in the liver, especially when calculated on the basis of blood glucose specific activity, suggests that pyruvate is perhaps more directly involved than glucose.

It is not possible to draw further conclusions about the details of the formation of inositol from glucose from the data *in vivo* presented here. It appears to be a fairly rapid process. The specific activity of the free tissue inositol found is of the order of 1% of that of blood glucose at 90 min after injection but clearly the precursors appeared at an earlier point in time and no information is available about their nature or the size of the pools involved. In addition, inositol is not only synthesized, but also undergoes further metabolism which has been studied extensively[47,55-65]. Apart from its incorporation into lipids it can give rise to glucose[55-57,59,60], presumably *via* glucuronic acid[47] and the pentose pathway[66], and thus is funneled off into multiple metabolic paths.

Yet no satisfactory explanation exists for the relatively large tissue concentrations. Despite some studies on and much interest in the role of both free inositol and phosphoinositides, the function of neither has to date been definitively elucidated. Free inositol is required as a growth factor in most strains of mammalian cell cultures[67], although at least one such strain is not inositol-dependent and can synthesize inositol from glucose[68]. Inositol has recently also been implicated in the nucleic acid metabolism of tissue-culture cells[69] and in the biosynthesis of cell walls in yeast[70].

As for the phosphoinositides, reviewed in 1960 by HAWTHORNE[5], their function is similarly obscure. The intriguing observations of HOKIN and his group that ^{32}P incorporation into the inositol phosphatides of secretory tissue slices is greatly increased upon stimulation[29] and its relation to active transport phenomena remain without adequate explanation.

In brain slices this increase was paralleled by an increased incorporation of inositol but not of glycerol[71]. The inositol phosphate portion of the molecule may therefore represent the functional end, although the glycerol "backbone" is renewed at an appreciable rate under physiological conditions. This is indicated by the data on phosphatide glycerol (Fig. 4 and Table V). Since inositides are formed by a different pathway from other glycerophosphatides, the glycerol in the individual molecular species may have different specific activities; the data are included here merely to point up the fact that the specific activities of lipid glycerol are of the same order of magnitude as of inositol, that they are also higher in young animals than in adults and that the carbonyl carbon of pyruvate is incorporated.

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