METABOLIC FATE OF BIOTIN AND OF AVIDIN-BIOTIN COMPLEX UPON PARENTERAL ADMINISTRATION

by

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Avidin has been shown to be the protein responsible for the ability of raw egg white to bind biotin and thus render dietary biotin unavailable[1,2]. In contrast to the effectiveness of avidin to cause egg white injury upon oral administration, its injection was shown to have a curative effect on the same deficiency disease[3]. This was explained on the basis of the fact that avidin always contains some biotin (corresponding to 10–20% of its capacity) and that this bound biotin was presumably available to the mammalian organism when given parenterally. All attempts to elucidate the mode of liberation of biotin from the complex, however, were unsuccessful[4]. The complex appeared remarkably resistant to all crude or purified enzyme systems studied, as well as to wide variations of p_H; only oxidative agents seemed to liberate small amounts of biotin.

The availability to us of avidin-biotin complex containing radioactive biotin[5] appeared to supply a new tool to investigate this problem. Since a tracing of the metabolic fate of biotin could hope to be successful only if relatively large amounts of radioactivity and thus of biotin were given (at least 0.01 mg or 0.04 microcurie), pilot experiments with similar amounts of nonradioactive biotin were first conducted. It appeared that in the rat, as in man[6], administration of superphysiological doses led to marked increases in urinary excretion of biotin within the first 24 hours. The same was true for radioactive biotin, about 40% of which appeared unchanged in the first day's urine; most of the residual radioactivity was also largely excreted at that time, although not as intact biotin. In contrast, biotin administered in the form of the avidin complex appeared in the urine at a more protracted rate, a total of about 50–60% being excreted within 5 days. No evidence for protein-bound biotin was found. The liver contained approximately 7% of the administered dose at the time of autopsy, but the residual radioactivity was not concentrated to such an extent in any of the other tissues or excreta investigated that it could be determined quantitatively.

This study has supplied the first incontrovertible evidence that biotin bound to avidin is released under physiological conditions and then follows a path similar to that of free injected biotin. This finding lends independent support to the seemingly paradoxical finding of GYÖRGY AND ROSE that avidin, or rather the complex, upon parenteral

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administration may cure the symptoms of egg white injury. The present study unfortunately has not revealed any clear evidence concerning the site or mechanism of release of the biotin.

EXPERIMENTAL MATERIALS AND METHODS

Radiobiotin, labelled with C^{14} in the ureido group, was kindly supplied by Dr D. B. MELVILLE of Cornell University. The avidin-biotin complex was material which had been used for equilibrium studies[6]. It was prepared from about 90% pure avidin NA[7,8] saturated with radioactive biotin of varying specific activity and dialyzed for protracted periods of time so that it was free from excess biotin or radioactivity.

Approximately 300 g rats were injected intraperitoneally with 4–5 ml solution containing 13.5 μg radioactive biotin (equivalent to 0.063 μc) or radiobiotin-avidin complex containing about 2 mg avidin and 13.0–15.4 μg radioactive biotin (equivalent to 0.038–0.062 μc) and immediately placed in a glass respiration chamber supplied with CO_2-free air. The urine was collected under toluene, feces in a wire trap, and expired CO_2 in 2 *N* sodium hydroxide.

Radioactivity was measured in a Geiger-Müller counter with thin mica window. Aluminum discs were employed in the preparation of plates from aliquots of urine or fractions thereof, of fractions obtained from liver, or of acetone slurries of homogenized tissues or feces. Small glass cylinders with ground edges were coated inside with a thin film of Dow Corning Silicone, then were rigidly held in place over the aluminum discs while the aliquots of urine (or other samples) were dried under an infra-red heat lamp. Barium carbonate precipitates from the alkali containing the expired CO_2 were plated from alcohol suspensions according to standard technique.

Aliquots of 3–5 ml urine were used in the preparation of plates. Since much of the radiation was expected to be absorbed by the bulky residue of the urine and since the specific activity of the 3 and 5 ml aliquots did not agree, experiments were carried out to determine the amount of self-absorption incurred by 3 and 5 ml nonradioactive urine containing known amounts of added radiobiotin. The necessary correction values were thus obtained: 3.0 and 1.9 for 5 and 3 ml urine samples respectively, independent of the biotin concentration. These were so high that the corrected values listed in Table I must be considered only as approximations. A similarly high correction value (2.5) had to be used in the case of acid hydrolysates of tissue which were neutralized directly on the plate with ammonia. In order to avoid this correction, the sulfuric acid was neutralized with baryta and the barium sulfate removed by centrifugation before the aliquots were taken for determination of radioactivity. Skepticism should also be entertained in the evaluation of the plates obtained from acetone slurries of whole tissue or feces, although no attempt was made to determine the amount of self-absorption in these cases due to the wide range in weight of the samples and the scarity of radiobiotin.

Biotin assays were kindly performed by Miss N. SNELL of the Western Regional Research Laboratory, using *Saccharomyces cerevisiae* F. B., in principle by the method of HERTZ[9].

RESULTS

Excretion of radioactive material

Urine analyses (see Table I). Microbiological assays indicated that the daily excretion of biotin was increased from a control value of 2 μg to 9 μg during the first 24 hour period after administration of 16 μg free biotin and then dropped back to the control value. The excretion of radioactivity during the first 24 hours corresponded to about 13 μg of radiobiotin, although the accuracy of this value is somewhat doubtful owing to the great corrections for self-absorption that had to be applied. To ascertain how much of this excreted radioactive material was unchanged biotin, excess avidin was added to aliquots of the urine which were then dialyzed. These experiments indicated that about half of the total radioactivity contained in the urine was in the form of avidin-combinable, and thus unchanged, biotin, in agreement with the microbiological assay value*.

* The radiobiotin as available to us contained about 2% nonbiotin material[5]; this insignificant contamination could not appreciably affect the present results.

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TABLE I

FATE OF RADIOBIOTIN AND RADIOBIOTIN-AVIDIN COMPLEX UPON PARENTERAL ADMINISTRATION

		Biotin	Biotin-avidin complex	
			I	II
Dose administered	μc	0.063	0.062	0.038
	μg[1]	15.6	15.4	13.0

PERCENTAGES OF ADMINISTERED RADIOACTIVITY[2]

	Total[3]	Biotin[4]	Total[3]	Biotin[4]	Total[3]	Biotin[4]
Urine Day 1	85 (3)	39 (7)	20 (1)		14 2)	10 (1)
2	1 (2)		22 (2)	23 (1)	18 (3)	19 (4)
3	1 (1)		4 (2)		13 (2)	14 (1)
4			2 (1)		6 (1)	
5			2 (1)		7 (1)	
6			1 (1)			
	87		51		58	
	Total	Biotin	Total	Biotin	Total	Biotin
Liver	4 (1)[5]	7 (2)[7]	7 (2)[5]		10 (1)[5]	9 (1)[7]
	5 (1)[6]		6 (3)[6]		8 (2)[6]	

1. Expressed as biotin.
2. Number of determinations in parentheses.
3. Total count expressed as percent of administered dose, obtained by plating urine directly and correcting for selfabsorption of 5 and 3 ml samples.
4. Amount of radiobiotin in urine capable of combining with avidin, expressed as percent of administered dose.
5. Total count expressed as percent of administered dose, obtained by plating acetone homogenates, thus only rough approximations.
6. Total count expressed as percent of administered dose, obtained by plating sulfuric acid digest.
7. Amount of radiobiotin in liver liberated by sulfuric acid and capable of combining with avidin, expressed as percent of administered dose.

After the administration of similar amounts of biotin complex-bound to avidin, rises in total biotin excretion above the control value of 2 μg per day were observed for 2–3 days. Three μg per day was the maximum observed. The excretion of radioactivity similarly extended over several days and amounted to 51–58% of the dose. In contrast to the results described above for the amount of true biotin compared with total radioactivity excreted by the rat receiving radiobiotin, the results obtained with the rats receiving avidin-radiobiotin complex would indicate that the total excretion of radioactive material was in the form of true biotin. Thus at least 50% of the radioactivity was dialyzable prior to the addition of avidin, but none after. The total amount of radiobiotin excreted by these rats was considerably more than the "extra" biotin excreted, as measured by microbiological assay, compared with the pre-injection control level, but was less than the total biotin appearing in the urine. Thus it would appear

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that the injection of a large dose of biotin (an eight-fold of the daily excretion value) depresses the normal mechanism of resorption from the intestine.

Preliminary studies of the effect of nonradioactive biotin (11 μg) and complex on the excretion of biotin by fasted rats indicated the same facts. Free biotin produced a markedly increased biotin excretion during the first day (the fasted control level of 1.0 μg per day was raised to 3.0 μg); whereas the complex effected an increase extending over 3 days (1.8, 2.2, and 1.4 μg per day respectively).

Expired CO_2. No count was obtained on the barium carbonate plates; it was therefore concluded that the injected biotin was not oxidized, or only to such a small extent as to be undetected in the Geiger-Müller counter.

Feces. Plates of acetone slurries of feces would indicate that approximately 7% of the total count was excreted in 3 days following the injection of radiobiotin and about 3% of the total count was excreted in the same time following the injection of avidin-radiobiotin complex.

Distribution of radioactivity in the body.

A qualitative search for radioactivity in various organs by direct plating of acetone suspensions of tissue homogenates indicated appreciable count only in the liver and considerably less in the kidney and spleen, with no radioactivity being observed in adrenal, mesentery, lung, blood, thyroid, and muscle. Acid extracts of the skin seemed to indicate a small amount of radioactivity to be present; however, the values obtained were so low and erratic that no definite conclusions could be drawn.

The nature and amounts of the radioactive material in the liver was investigated further after acid hydrolysis of samples of the tissue. The most reliable analyses were probably those in which the sulfuric acid was removed by neutralization with baryta. These indicated that 5–8% of the administered radioactivity was in the liver after 3–6 days, apparently largely as biotin, since most of it was rendered nondialyzable upon the addition of avidin (see Table I).

Liver tissue was also fractionated by the method of HOFFMANN[10], as well as by an ammonium sulfate fractionation procedure designed to reveal the presence of radiobiotin in a fraction corresponding in solubility to avidin or complex (see Table II). The former

TABLE II

FRACTIONATION OF LIVER

Experimental conditions	Free radiobiotin		Avidin-radiobiotin complex
	% of dose[1]	μg biotin per g liver[2]	% of dose[1]
Hoffmann technique			
Acid extract	0.9	1.3[3]	0.7, 1.5
Alkaline extract	3.4	2.1[4]	3.2, 2.6
Ammonium sulfate			
0.5 satd. AS			4.6
1.0 satd. AS			0
Satd. AS supernatant			0.6

1. As calculated from radioactivity.
2. By microbiological assay.
3. Active without prior hydrolysis.
4. Only 10% as active without hydrolysis.

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method yielded results concerning the fractionation of liver biotin which confirmed HOFFMANN well. The liver contained 3.4–4 μg biotin per g dry tissue, which was about 10 times its content of radiobiotin. The radioactivity appeared in both the acid and alkaline extracts, approximately in proportion to their total biotin contents.

The ammonium sulfate fractionation yielded the highest count in the globulin fraction, and no detectable amount in the fraction precipitated above 2.0 *M* ammonium sulfate, which would contain any avidin or complex. There was a small amount of non-dialyzable activity not precipitated with ammonium sulfate.

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SUMMARY

1. The parenteral administration of 0.01–0.02 mg biotin to fed or fasted rats caused a marked increase in biotin excretion for one day, accounting for up to 40% of the dose.

2. The use of labelled biotin in a similar experiment indicated that most of the residual biotin was also excreted within one day although not in microbiologically active form.

3. Of similar amounts of radiobiotin administered in complex form with avidin, about one half was excreted over a 3–5 day period, apparently largely as free biotin.

4. In each experiment, a small fraction of the administered radiobiotin was found in the liver, but no appreciable amount in the other organs tested.

RÉSUMÉ

1. L'administration parentérale de 0.01–0.02 mg de biotine à des rats alimentés ou à jeûn causa une augmentation considérable de l'excrétion de biotine pendant un jour, atteignant 40% de la dose.

2. L'emploi de biotine marquée montra, dans une expérience semblable, que la majeure partie de la biotine résiduelle était également excrétée pendant un jour, quoique non sous une forme microbiologiquement active.

3. Environ la moitié de quantités semblables de radiobiotine administrées sous forme de complexe avec l'avidine, fut excretée pendant une période de 3 à 5 jours, apparemment en majeure partie sous forme de biotine libre.

4. Dans chaque expérience une petite fraction de la radiobiotine administrée fut trouvée dans le foie; aucune quantité appréciable ne fut trouvée dans les autres organes étudiés.

ZUSAMMENFASSUNG

1. Die parenterale Verabreichung von 0.01–0.02 mg Biotin an gefütterte und nicht gefütterte Ratten verursachte eine bedeutende Zunahme der Biotinausscheidung während eines Tages, welche bis zu 40% der Dosis entsprach.

2. Die Anwendung von markiertem Biotin in einem ähnlichen Versuche zeigte, dass der grösste Teil des restlichen Biotins auch während eines Tages ausgeschieden wurde, allerdings nicht in mikrobiologisch aktiver Form.

3. Wurden ähnliche Mengen von Radiobiotin in Form eines Komplexes mit Avidin verabreicht, so wurde ungefähr die Hälfte während einer Zeitspanne von 3 bis 5 Tagen ausgeschieden, anscheinend in Form von freiem Biotin.

4. In jedem Versuch wurde eine kleine Menge des verabreichten Radiobiotins in der Leber gefunden; in den anderen untersuchten Organen wurden keine merklichen Mengen nachgewiesen.

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