(see Table 2). Both $R_g$ and $R_T$, which are obtained from spacecraft data, seem to be systematically smaller than the radii $R_e$ obtained from what is probably the best earth-based data (*3*). The root-mean-square value of $R_e$ is approximately 2.5 km larger than that of both $R_g$ and $R_T$. The Ranger and Orbiter data (*4*) also seem to be systematically 2.5 km smaller than earth-based data.

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## Brain Serotonin Content: Increase Following Ingestion of Carbohydrate Diet

Abstract. *In the rat, the injection of insulin or the consumption of carbohydrate causes sequential increases in the concentrations of tryptophan in the plasma and the brain and of serotonin in the brain. Serotonin-containing neurons may thus participate in systems whereby the rat brain integrates information about the metabolic state in its relation to control of homeostatis and behavior.*

The concentration of serotonin, a putative neurotransmitter in the mammalian central nervous system, varies as a result of physiological changes in the availability of its precursor, tryptophan (*1, 2*). Large parenteral doses of tryptophan rapidly increase the amount of serotonin in the brain (*3*). Increases (*4*) or decreases (*5*) in dietary tryptophan over several weeks or more are associated with parallel alterations in brain serotonin. Tryptophan concentrations in plasma and brain (*1, 2, 6*) and serotonin concentrations in brain (*7*) undergo characteristic daily variations. If rats are injected with small amounts of tryptophan (less than one-twentieth of their daily dietary intake) at the time of day when tryptophan concentrations in the brain and plasma are lowest, the serotonin in the brain increases within about an hour (*2*). Brain tryptophan also increases, but remains within the normal daily range (*6*). The foregoing observations suggest that naturally occurring changes in plasma tryptophan (for example, in response to diet) could "drive" brain tryptophan concentrations, and that changes in the latter could, in turn, affect brain serotonin.

Administration or secretion of insulin lowers the concentrations of glucose and of most amino acids in the plasma (*8, 9*). In contrast, tryptophan in plasma *increases* in rats injected with insulin or given access to a carbohydrate diet (*9*). Thus it might be anticipated that insulin would also increase the concentrations of brain tryptophan, and, ultimately, of serotonin. Very high doses of insulin have been shown to elevate brain serotonin concentrations (*10*); however, because these doses also produced convulsions, the change in brain serotonin could have been due to abnormal neuronal activity, and not to enhanced concentration of tryptophan in the brain.

We now show that in rats the increase in plasma tryptophan after ingestion of carbohydrate or after injection of subconvulsive doses of insulin is accompanied by parallel increases in the concentrations of tryptophan and serotonin in the brain.

Male Sprague-Dawley rats (Charles River Laboratories) were housed five per cage, exposed to light (*11*) from 9 a.m. to 9 p.m. daily, and given free access to food (Big Red Laboratory Animal Chow, Agway) and water. At 9 p.m. on the evening before an experiment the rats were placed in clean cages and deprived of food. Between noon and 3 p.m. the next day, groups of seven animals were injected intraperitoneally with insulin (2 units of Iletin per kilogram of body weight, 40 unit/ml) diluted in water. During this interval other uninjected groups of ten animals were given free access to a protein-free diet composed largely of carbohydrates (*12*). Rats were decapitated 1, 2, or 3 hours after injection or exposure to the diet, and blood from the cervical wound was collected in heparinized tubes and centrifuged. Plasma was frozen and subsequently assayed for tryptophan (*13*), tyrosine (*14*), and glucose (*15*). The brains were quickly removed, bisected midsagittally, and frozen on Dry Ice. One-half of each brain was assayed for tryptophan (*13*), and the other half was assayed for serotonin (*16*). In no case did the levels of compounds measured in control animals at 1, 2, or 3 hours after the start of an experiment differ from those at zero time. Hence all comparisons between control and insulin-treated rats were made with the zero-time control group as base.

As shown earlier (*9*), plasma tryptophan concentrations in rats receiving insulin were increased by 40 percent as compared to those of control animals ($P < .01$) 2 hours after injection

Table 1. Effect of carbohydrate ingestion on brain serotonin concentrations and on plasma and brain tryptophan. Plasma amino acid concentrations are in micrograms per milliliter. Brain tryptophan and serotonin concentrations are in micrograms per gram of brain, wet weight. The average animal weight in experiment 1 was 160 g. The average animal weight in experiment 2 was 260 g.

| Time after presentation of food (hours) | Tryptophan | | Serotonin in brain ($\mu$g/g) | Tyrosine in plasma ($\mu$g/ml) |
|---|---|---|---|---|
| | Plasma ($\mu$g/ml) | Brain ($\mu$g/g) | | |
| | | *Experiment 1* | | |
| 0 | 10.86 ± 0.55 | 6.78 ± 0.40 | 0.549 ± 0.015 | 13.03 ± 0.29 |
| 1 | 13.56 ± 0.81* | 8.32 ± 0.63† | 0.652 ± 0.046 | 9.55 ± 0.34‡ |
| 2 | 14.51 ± 0.70‡ | 11.24 ± 0.52‡ | 0.652 ± 0.012‡ | 8.67 ± 0.26‡ |
| 3 | 13.22 ± 0.65* | 9.81 ± 0.50‡ | 0.645 ± 0.017‡ | 9.03 ± 0.21‡ |
| | | *Experiment 2* | | |
| 0 | 15.09 ± 0.39 | 3.32 ± 0.16 | 0.60 ± 0.01 | 14.23 ± 0.66 |
| 1 | 15.79 ± 0.49 | 4.70 ± 0.12‡ | 0.75 ± 0.01‡ | 10.69 ± 0.23‡ |
| 2 | 16.65 ± 0.52† | 5.77 ± 0.09‡ | 0.76 ± 0.01‡ | 10.06 ± 0.24‡ |
| 3 | 15.26 ± 0.50 | 6.29 ± 0.21‡ | 0.76 ± 0.02‡ | 11.17 ± 0.40‡ |

* $P < .02$ differs from zero-time group; † $P < .05$ differs from zero-time group; ‡ $P < .001$ differs from zero-time group.

(Fig. 1), while plasma glucose decreased significantly (Fig. 1). Brain tryptophan and serotonin also increased after insulin administration, and attained, within 2 hours, values 36 percent ($P < .001$) and 28 percent ($P < .01$) above those of control animals (Fig. 1).

To determine whether the changes in brain tryptophan and serotonin that followed insulin administration also occurred in response to the physiological secretion of insulin, we examined plasma tryptophan and brain tryptophan and serotonin in rats that had been fasted and then allowed to eat a protein-free, largely carbohydrate diet for 1, 2, or 3 hours. (This diet was used in order to exclude a dietary source of tryptophan.)

In the first experiments, the animals ate about 5 g/hr during the first hour, and 2 g/hr during the second and third hours. Tryptophan in the plasma, as compared to that of control rats killed at the beginning or the end of the experiment, significantly increased

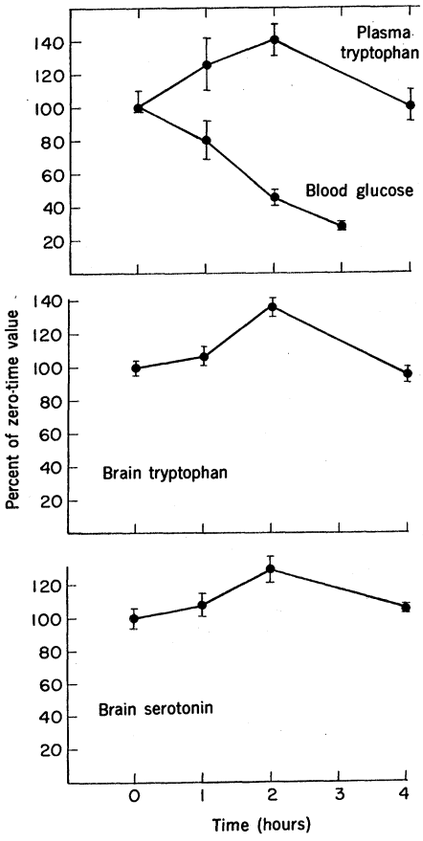


Fig. 1. Effects of insulin administration (2 unit/kg, intraperitoneally) on plasma tryptophan, blood glucose, brain tryptophan, and brain serotonin concentrations. Groups of six to ten rats were killed at 1-hour intervals after insulin was administered. Vertical bars indicate standard errors of the mean.

1, 2, and 3 hours after the food was first presented. Tyrosine concentrations in the plasma were depressed at all times (Table 1). Brain tryptophan concentrations rose 22 percent during the first hour, attained a peak 65 percent above control values ($P < .001$) 2 hours after food presentation, and remained significantly elevated at 3 hours (Table 1). Brain serotonin concentrations also rose during the first hour, but did not become significantly elevated (19 percent over control levels, $P < .001$) until 2 hours after the initial presentation of food. They remained elevated at 3 hours.

In a second, similar experiment performed with larger rats (260 g compared to 160 g), plasma tryptophan did not significantly increase until 2 hours after food was presented, and then increased to only 10 percent above control values. But plasma tyrosine still decreased dramatically, reaching a minimum (29 percent below control values) after 2 hours. Brain tryptophan steadily increased throughout the feeding period, and 3 hours after the food was presented it was 90 percent above that in the controls. Brain serotonin significantly increased (25 to 27 percent above control, $P < .001$) at all three times examined. In this second experiment, the rats consumed about 8 g of food during the first hour, and 3 g during the second and third hours.

Thus the administration of insulin or the ingestion of a largely carbohydrate diet increases the amount of tryptophan in plasma and brain, but decreases the amounts of tyrosine (Fig. 1) (Table 1) and most other amino acids in the plasma (*9*). This increase in brain tryptophan is associated with a parallel increase in the concentration of brain serotonin. These effects are not due to severe hypoglycemia, since they occur in rats whose plasma glucose presumably is elevated by the consumption of a high-carbohydrate diet (Table 1). The results also cannot be attributed to glucagon secretion because administration of glucagon does not cause an increase in plasma tryptophan (*9*).

The source of the additional tryptophan in plasma after insulin administration or secretion has not yet been identified, nor has the mechanism by which insulin increases brain tryptophan been established. Insulin could produce the latter effect in any of the following ways. (i) It may enhance the diffusion of the amino acid along a concentration gradient as its plasma concentration rises. This is probably not the sole mechanism operating, since, in our experiments, the increases in concentrations of tryptophan in the brain tended to be greater than those in plasma. (ii) It may decrease the proportion of plasma tryptophan that is bound to albumin (*17*) and thereby increase the size of the pool of diffusible, or free, tryptophan that presumably is in equilibrium with tissue tryptophan. (iii) It may exert a direct, stimulatory effect on the net uptake of tryptophan into the brain. (iv) It may cause the release of other factors or hormones (such as corticosterone) that might produce one or more of the above effects. Insulin also might increase the tryptophan in the brain by decreasing the utilization of the amino acid. This hypothesis is not supported, however, by the evidence (*10*) (Fig. 1) that the concentration and turnover of serotonin, a major tryptophan product, increases in brains of animals receiving insulin. These increases in brain serotonin most likely reflect an acceleration in its synthesis (*1, 2, 18*). As the concentration of brain tryptophan rises, the percent saturation of tryptophan hydroxylase, the enzyme that catalyzes the initial step in serotonin biosynthesis (*19*), probably also increases.

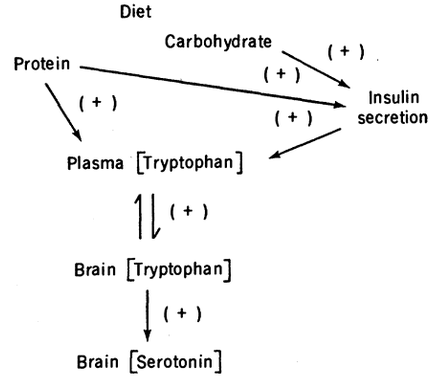


Fig. 2. Proposed sequence describing the mechanism by which food consumption elevates brain serotonin concentration in the rat. The carbohydrate, protein, and, probably, fatty acid (*20*) constituents of the diet elicit insulin secretion; this causes an increase in plasma tryptophan (*9*) (Fig. 1); dietary protein also elevates plasma tryptophan by directly contributing to it (*21*). The carbohydrate-induced increase in plasma tryptophan causes a parallel rise in brain tryptophan (*2*) (Fig. 1), thus enhancing the substrate saturation and the activity (*19*) of the tryptophan hydroxylase in serotonin-producing neurons (*22*). The rate of serotonin synthesis is thereby accelerated, ultimately raising the concentration of serotonin in the brain.

This enzyme has an unusually high $K_m$ (Michaelis constant) [$3 \times 10^{-4} M$ (*19*)] for tryptophan, and therefore its activity in vivo probably depends on the concentration of tryptophan. If our measurements of tryptophan concentrations in whole brain are indicative of those actually within serotonin-producing cells, then the increase in brain tryptophan produced by insulin would be of sufficient magnitude to cause a considerable acceleration in serotonin synthesis. In support of this hypothesis, we have shown (*2*) that small increases in tryptophan concentration in brain which occur after the injection of 12.5 mg of tryptophan per kilogram of body weight also cause significant elevations in brain serotonin (*2*).

Since almost any food that the rat might consume would probably elicit insulin secretion and thereby elevate plasma tryptophan, our observations suggest the sequential relation between food consumption and brain serotonin content described in Fig. 2. Carbohydrates, proteins, and, probably, fats (*20*) elicit insulin secretion in various mammalian species; dietary proteins would also be expected to raise plasma tryptophan by a direct contribution (*21*). It remains to be determined whether plasma and brain tryptophan, and brain serotonin, are also elevated in rats consuming diets other than pure carbohydrate.

Serotonin-containing neurons apparently participate in the control of a variety of behavioral and neurovisceral functions, including sleep, thermoregulation, motor activity, food consumption, and the secretion of hormones from the anterior pituitary gland. The increase in brain serotonin that occurs in rats given tryptophan appears to be highly localized to these serotonin-containing neurons (*22*). If the insulin-induced changes in brain serotonin content are, in fact, associated with alterations in the input-output characteristics of serotonin-containing neurons, then the sequence described in Fig. 2 may represent an important component of the systems by which the rat brain integrates information about the metabolic state of the animal into its control of homeostasis and behavior.

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## Subhuman Primate Diploid Cells: Possible Substrates for Production of Virus Vaccines

Abstract. *Results of a program for development of new cell lines suggest that it it possible to establish cell lines from both rhesus and African green monkeys which are comparable to diploid lines of human origin, and that these monkey lines should be candidates for use in the production of virus vaccines.*

Most of the virus vaccines licensed for human use in the United States are prepared from primary cell cultures derived from monkeys, chicks, ducks, or rabbits (*1*). All of these tissue sources, except for monkeys, come from closed breeding colonies that are free of specific pathogens. Monkeys whose tissues will be used for vaccine production are held in quarantine for at least 6 weeks, but cross infection among these animals can occur, and their use poses many practical problems as well as certain theoretical risks.

The clinical experience with virus vaccines produced from monkey kidney cell cultures has been overwhelmingly successful. Nevertheless, it would be better to produce vaccines in a cell substrate which had been extensively studied and characterized as being free of any known microbial agent. The suggestion that vaccines be produced in a well-characterized cell system such as WI-38, a diploid cell line of human origin, instead of in primary cultures was first made in 1961 (*2*), and this recommendation has been offered repeatedly since then (*3*). This report describes the results of a program for development of diploid cell lines from subhuman primates. In these results, several cell lines appear promising for use in production of vaccines.

The published data on WI-38 cells has indicated the general safety and economic practicability of usage of this diploid cell line. However, there have been reservations about the use of parenterally administered vaccines made from such cells. Because of these theoretical objections to the use of human cells in vaccine production, the Division of Biologics Standards (*4*) felt that alternatives to WI-38 should be explored. Priority was given to de-