cent tolerance in C57B1/6J females by neonatal injections of viable isologous male spleen cells. The cells were injected into the facial vein; mice under 12 days of age received 5 to 10 million spleen cells and slightly older mice received 12 to 20 million cells. When the immunized females were 8 weeks old, they were grafted with isologous male skin. Mice first injected with male spleen cells after 17 days of age exhibited an accelerated rejection rather than delayed rejection of the male skin graft. Mariani *et al.* (*4*), using the same strain of mice, were able to induce tolerance to male skin (grafted 30 days after injection of cells) by a single intravenous injection of 20 million spleen cells into females of 32 to 48 and 69 to 91 days of age. The former gave 100 percent tolerance and the latter 80 percent. They also found that parabiotic union between adult male and female mice of the same strain induced tolerance to male skin in the female partner.

By using sperm as a source of the so-called Y chromosome antigen, we have shown that injection of sperm cells into C57B1/6J females can result in accelerated rejection, delayed rejection, or tolerance, depending upon the dose of sperm injected and the subsequent time of grafting with isologous male skin.

Untreated adult C57B1/6J females tolerated isologous male skin for a median time of 28 days and a range of 25 to 31 days (Table 1, group 4). When, however, females of this strain were injected intraperitoneally with 1 to 2.5 million isologous sperm cells, 5 to 14 days prior to grafting, the rejection time of the graft was reduced to 15 $\pm$ 3 days (groups 5 to 7). On the other hand, females of this strain injected intraperitoneally with 4 million sperm cells 5 days before grafting retained isologous male skin for 27 $\pm$ 3 days (group 8). Further, at a dose level of 8 million sperm cells given 5 or 14 days before transplantation, such females retained grafts for more than 100 days or until termination of the experiment (group 9). Females injected with 1 million sperm cells 21 days prior to application of the male graft have exhibited graft maintenance for at least 60 days and are still under observation at this writing.

The data in Table 1 indicate that large doses of antigen result in tolerance to isologous male skin and that small doses of antigen produce accelerated rejection if the graft is applied within 2 weeks. Smaller doses may also lead to tolerance if the graft is not applied until later. The latter indicates that tolerance may be preceded by a period of accelerated rejection.

This result is similar to the finding of Crowle (*5*) that mice made sensitive to egg albumin may be made tolerant to this antigen by a course of desensitization. In his experiments, the long term tolerance was preceded by a period of several weeks of rebound in the sensitivity. Both his and our results are consistent with the hypothesis that tolerance is produced by forced maturation of the stem cells which are the locus of specific immunologic memory. According to this hypothesis (*6*), maturation results in synthesis of specific protein for the life span of the mature cell, but loss of the replicative ability of the stem cell on which immunologic memory depends.

In addition, these data indicate that sperm are richer in Y chromosome antigen than spleen cells. The injection of 5.5 million male spleen cells produced accelerated rejection of a male graft applied 5 days later (group 11) in contrast to the first evidence of maintenance produced by only 4 million sperm (group 4). On the basis that increased injection of antigen leads to tolerance rather than accelerated rejection, this would indicate that there is more antigen per sperm cell than per spleen cell. Since the spleen cell is probably at least ten times larger, the sperm cell must contain a considerably higher concentration of antigen.

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Intestinal Invertase: Precocious Development of Activity after Injection of Hydrocortisone

Abstract. *Injection of rats aged 3 to 9 days with hydrocortisone causes precocious development of invertase activity in the small intestine. The enzyme becomes fully active about 72 hours after injection of hydrocortisone. Invertase activity is also detectable when hydrocortisone is added to organ culture of intestine derived from 5- to 6-day old rats. Hydrocortisone does not appear to affect the activity of lactase, suggesting that it does not act solely by hastening the normal maturation process.*

In man, two nutritionally important intestinal disaccharidases, lactase and invertase, are active at birth (*1*). In pigs, rats, and mice there is a dissociation of these two activities; lactase is fully active at birth, but the activity of invertase is not detectable until sometime later in development (*2*, *3*). Both of these enzymes are primarily localized in the jejunum, and about 90 percent of the invertase is associated with the large granule fraction obtained by centrifugation; the remainder sediments with the microsomes (*3*). Miller and Crane (*4*) have proposed that invertase is contained within or upon the brush-border membrane of the intestinal mucosal cell, since such membranes are morphologically identifiable in large granule fractions of homogenates.

A duodenal alkaline phosphatase shows a developmental pattern of activity similar to invertase and also is localized in the brush border (*5*). Furthermore, this enzyme is active much earlier if cortisone is injected. We, therefore, studied the effect of hydrocortisone on the activity of invertase in the intestine of the young rat. Injections of hydrocortisone cause the enzyme activity to be detectable at a much earlier stage in development. This phenomenon represents direct action of hydrocortisone on the intestinal mucosa, since invertase can be detected in small pieces of young intestine cultured in vitro in the presence of hydrocortisone.

Wistar rats aged 9 days were injected intramuscularly with hydrocortisone (50 mg/kg) and were killed at daily intervals thereafter. Controls were injected with the vehicle in which the drug was administered, or were untreated. The jejunum was removed, rinsed in cold 0.15*M* KCl and homogenized in

ten volumes of cold 0.65*M* mannitol. Enzymatic activity was measured in 0.1*M* acetate buffer, *p*H 6.0, in a total volume of 2.0 ml containing 0.25*M* sucrose and 40 μmole of iodoacetate to inhibit glycolysis. Samples were incubated at 37°C for 10 minutes and the reaction was stopped by heating at 100°C for 1 minute. The glucose released from sucrose was determined with glucose oxidase reagent (*6*). Protein was measured by the method of Lowry *et al.* (*7*).

Eagle's basic medium supplemented with 10 percent calf serum and 50 units each of penicillin and streptomycin per milliliter was used for organ culture. Pieces of jejunum, approximately 2 cm long, were slit and rinsed in culture medium containing ten times the standard concentration of penicillin and streptomycin. They were given a second rinse in the standard medium and were then placed on individual "boats" consisting of a piece of filter paper approximately 1 × 3 cm fused by autoclaving to a plastic frame. The volume of culture medium was adjusted so that the tissue remained about half immersed in medium. The gas phase consisted of 95 percent oxygen and 5 percent carbon dioxide, and it was renewed after 16, 48 and 96 hours of culture. Glucose was replenished, and the *p*H of the medium readjusted to 7.4, after 16 hours. Hydrocortisone-sodium succinate in sterile aqueous solution was added to appropriate flasks.

For assay, medium and tissue were transferred to centrifuge tubes, centrifuged at 600*g* for 15 minutes at 4°C, and the sediment washed twice with cold, isotonic KCl. The pellet was homogenized in 2 ml of 0.65*M* mannitol and assayed for invertase as described, except that the incubation time was increased to 30 minutes. Activity of lactase was measured as described previously (*8*).

Activity of invertase was detected within 24 hours in animals that were injected with hydrocortisone when 9 days old. The enzyme was fully active within 2 to 3 days (Fig. 1). After 5 days the activity began to decrease, and remained low until about the 9th day after injection (when the animals were 18 days old); the activity then increased again, closely following the curve for control animals. A similar pattern was observed when animals aged 5 days were injected with hydrocortisone; the hormone was capable of eliciting invertase activity in the intestine when the animals were as young as 3 days. When hydrocortisone was injected daily (25 mg/kg), after an initial injection of 50 mg/kg at 9 days of age, the enzyme was fully active at least until the animals were 21 days of age. As little as 5 mg/kg was sufficient to elicit enzymatic activity.

Table 1. Stimulation of invertase by hydrocortisone in organ cultures of rat intestine. Jejunum from 5- or 6-day-old rats was grown in Eagle's medium plus 10 percent calf serum, with or without the indicated amount of hydrocortisone (Solu-Cortef; Upjohn). The gas phase was 95 percent O_2, 5 percent CO_2; *p*H was adjusted after 16 hours in culture and glucose was replenished at the same time. The gas phase was renewed after 16, 48, and 96 hours of culturing.

Expt. No.	Time in culture (days)	Conc. hydrocortisone (*M*)	Lactase*		Invertase*	
			Control	Exptl.	Control	Exptl.
12	2	2×10^{-4}	1.9	1.9	0	0
11	3	1×10^{-4}	4.7	5.7	0	0.11
9	5	2×10^{-4}	1.5	3.6	0	0.48
		2×10^{-5}		2.5		0.40†
11	5	1×10^{-4}	2.3	2.2	0	0.28†
9	7	2×10^{-4}	4.7	4.0	0	0.21
		2×10^{-5}		2.1		0.16†

* Results are expressed as μmoles of glucose released per milligram protein per hour. † Assay of single flask, all others are average of duplicate flasks.

If adrenocorticotropic hormone was injected twice daily (40 U.S. Pharmacopeia units/kg) from either the 5th or 9th day after birth, a similar precocious appearance of invertase activity occurred following a latent period of 2 to 3 days (Fig. 2).

Morphologically, the intestine of these hydrocortisone- or ACTH-treated animals resembled that of a mature animal. The intestinal epithelium was more cellular, the glands were more complex and the villi were longer than in untreated controls of the same age. These changes are similar to those described by Clark (*9*) for intestine from rats treated with cortisone.

Activities of tryptophan pyrrolase, tyrosine transaminase and alkaline phosphatase have been increased by the addition of cortisone in vitro; the first two enzymes in liver perfusion systems (*10*), and alkaline phosphatase in cell lines grown in vitro (*11*). An attempt was made to stimulate the appearance of invertase with hydrocortisone in organ cultures of jejunum derived from 5- to 6-day-old rats. Invertase activity was not observed in tissue grown without hydrocortisone, but it was detected after 3 days of incubation in the presence of hormone. Under the conditions of these experiments there was no relationship between the concentration of

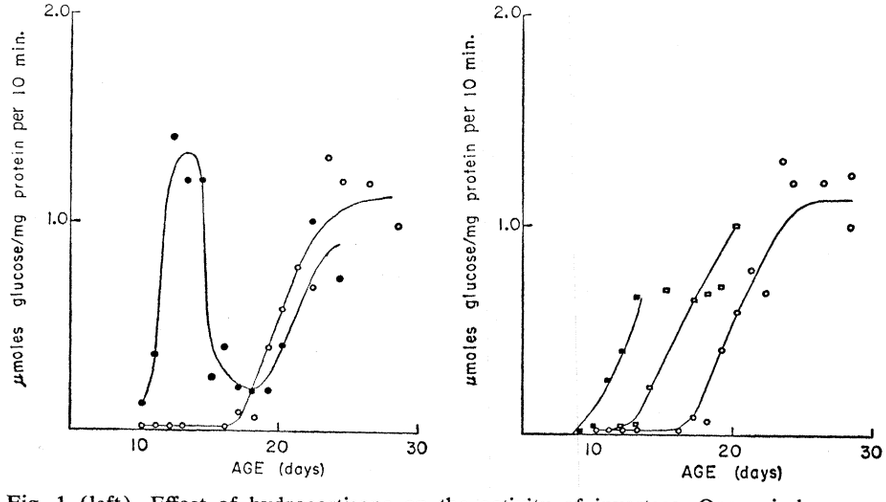

Fig. 1 (left). Effect of hydrocortisone on the activity of invertase. Open circles, controls. Solid circles, hydrocortisone injected intramuscularly (50 /mg/kg) at 9 days of age. Invertase activity is expressed as micromoles of glucose formed per milligram of protein per 10 minutes. Fig. 2 (right). Effect of ACTH on the activity of intestinal invertase. Open circles, controls. Open squares, ACTH injected twice daily (40 USP units/kg) from 9 days of age. Solid squares, ACTH injected twice daily from 5 days of age. Invertase activity is expressed as micromoles of glucose formed per milligram of protein per 10 minutes.

hydrocortisone and the production of invertase activity (Table 1).

Each organ culture was assayed for activity of lactase as a measure of physiological condition of the tissue. Values for activity of this enzyme were from 20 to 60 percent of the specific activity usually found in fresh intestinal mucosa from rats of this age. The tissue, therefore, maintained relatively normal enzymatic activity during the culture period. It would seem that the appearance of invertase in the intestine of the young rat is a result of hormonal action directly upon intestinal mucosal cells.

There was no decrease in activity of lactase compared with untreated controls, either in the intact intestine after administration of hydrocortisone, or in organ cultures grown in the presence of hydrocortisone. In fact, either normal or slightly elevated activity was found. If the effect of hydrocortisone on the activity of intestinal invertase was due to the maturation of the tissue, then a decrease in activity of lactase would have been expected (*8*). Thus, hydrocortisone does not seem to act solely by hastening normal maturational changes in the intestine. It must also act by a mechanism as yet unknown. This hormone-enzyme interrelationship should be a suitable system for studying the regulation of a developmental process (*12*).

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DNA Segregation in Escherichia coli: Observations by Means of Tritiated Thymidine Decay

Abstract. Escherichia coli, *strain 15T-L-, inactivation by H³-thymidine decay was used in a study of DNA segregation. Two characteristics of the inactivation curves due to H³-thymidine decay, the initial slope and stable fraction, enable the mode of segregation to be defined. The results suggest that the segregation pattern is consistent with a semiconservative model of DNA replication. The data on the stable fraction indicate that the DNA of strain 15T-L-, under our experimental conditions, may be organized into one duplex structure. Since colony-forming ability was used as the means of assay, it is possible to compare these experiments with those in which DNA segregation is examined on a structural basis.*

Since Hershey *et al.* (*1*) discovered that the decay of radiophosphorus following its incorporation into the DNA of bacterial viruses causes death of the viruses, the technique of "radioactive suicide" has become widely used as a tool in research (*2*). "Radioactive suicide" is taken as a measure of the destruction of functional biological units by accumulated radioactive decay. Fuerst and Stent (*3*) were the first to suggest that from such experiments one might obtain information about the segregation of DNA in bacteria. In a series of experiments with *Escherichia coli,* they obtained survival curves for cells which had been fully labeled with P^{32} and for cells that had been fully labeled and then allowed to grow for varying times in the absence of P^{32}. The assay used was one of colony-forming ability. Their data indicated that, under their experimental conditions, the segregation processes were not simple enough to permit the pattern of DNA segregation to be defined unequivocally.

Recently, it was shown (*4*) that H^3-thymidine, when incorporated into *E. coli* 15T-L-, a strain which requires thymidine and leucine, causes death by radioactive decay at a rate comparable with that for P^{32} decay. In contrast to P^{32}, H^3-thymidine is incorporated nearly exclusively into the bacterial DNA. Therefore, we studied the segregation of DNA in *E. coli* 15T-L-, using H^3-thymidine decay, using procedures similar to those of Fuerst and Stent (*3*). Our results suggest that the pattern of segregation is indicative of semiconservative DNA replication.

The pattern of DNA segregation may be understood from an analysis of the possible modes of replication for a one duplex or two stranded structure. As stated by Delbruck and Stent (*5*), the scheme is (i) conservative if the original duplex molecule remains intact and produces a completely new two-stranded molecule; (ii) semiconservative if the chains separate without breakage and each serves as the template for the formation of a complementary chain; (iii) dispersive if the chains are broken or destroyed in the process.

These models of DNA replication allow theoretical predictions with regard to the survival curves for fully labeled cells, and for fully labeled cells that have been allowed to grow for n generations in nonradioactive medium, if the mode of organization of DNA within the cell is of a simple nature. These theoretical predictions for cells containing one duplex structure are summarized in Table 1. For fully labeled cells the survival relation is the same for all three models and is $N/N_0 = e^{-\alpha\lambda N^* t}$ (symbols are defined in the title of Table 1). For fully labeled cells grown for n generations in cold medium the initial slopes for the conservative, semiconservative and dispersive models, when compared with that for the fully labeled cells should be in the ratio of 1, ½, $½^n$, respectively. In addition, for the conservative and semiconservative models the survival curves should show leveling at a point dependent both on n and on the model.

We made a brief examination of the survival of cultures of *E. coli* 15T-L-, which had been fully labeled with H^3-thymidine and then grown for various lengths of time in cold thymidine. We observed that the survival curves were similar in character to the semiconservative category in Table 1, indicating that, for this type of cell and for this