similar fashion. Those animals which showed prolonged clearance time of antigen injected both in the neonatal period and at 10 weeks and which showed no precipitating antibody after either injection, are considered to be tolerant(1a). Those producing circulating antibody after either injection are considered immune. By this grouping it is shown clearly that the amount of antigen injected initially, even though adjusted in amount for rapid growth of animals, was adequate to induce tolerance regularly only during the first 2 weeks of life, as previously shown for a single quantity of antigen. A marked qualitative change in animals' response to this heterologous protein, therefore, must have occurred between 14th day of life when 80% of the litters became tolerant and 21st day when only 27% failed to produce antibodies.

Discussion. Rabbits older than 28 days of age consistently produced antibodies to a single injection of BSA, while rabbits under 14 days of age handled this protein with no evidence of immune response. Rabbits between these 2 age groups responded variably, but most injected at 14 days of age were unresponsive while most 21-day-old animals had a primary immune response with production of precipitating antibody. These data again demonstrate that the period during which unresponsiveness can be induced is of finite length, consistent with a qualitative deficiency in the immune mechanism. These data also suggest strongly that different mechanisms probably underlie the model of unresponsiveness which is induced in adults by large amounts of antigen(3) and the type tolerance induced during the neonatal period.

Summary. Neonatal rabbits respond to a single injection of BSA in a dose of 200 mg/kilo body weight with antibody production when given at 28 days of age or older and are unresponsive when this weight-adjusted amount of BSA is given at 8 days of age or younger. At 14 and 21 days of age the response is varied and the majority injected at 14 days of age are unresponsive while most formed antibody when injected at 21 days of age.

The authors acknowledge the technical assistance of Earle Calahan, Marjorie Wright, and Billievelyn Miller.

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Received September 8, 1959. P.S.E.B.M., 1959, v102.

Inhibition of Cleavage and Development of the Sea-Urchin Egg by 2-Desoxy-D-Glucose.* (25308)

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An analogue of glucose, 2-desoxy-D-glucose (2DG), has been shown to be a competitive inhibitor of glucose metabolism in yeast(1,2) and rat tissues(3-6). It is also known to retard cell-division in chick heart fibroblasts cultured *in vitro*(7), and in tumors(8-10), and budding in yeasts(2). In view of above work, it appeared to us that it would be of interest to investigate the action of this inhibitor on respiration and development of fertilized eggs of sea-urchins. Both glycolytic and respiratory metabolism are required for cleavage of these eggs, since development is blocked by

* This investigation was supported by a research grant from Nat. Cancer Inst., N.I.H., P.H.S.

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fluoride and iodoacetate, as well as low O_2 tension, CO, and cyanide(11,12). At the same time, since in early development of the egg only endogenous nutrient is utilized, it has been difficult to specify what substrates are metabolized. Use of 2DG therefore should provide some information about the importance of glucose-metabolism to the dividing egg. The inhibitory effects of 2DG on cleavage, development and oxygen uptake of eggs from the sea-urchin *Strongylocentrotus purpuratus* are described here.

Methods. Eggs were obtained from excised ovaries and washed several times in filtered sea water before use. Eggs were mixed 15 minutes after fertilization with equal volumes of a solution of 2DG in spot plates or salt cellars which were sealed and stored at 16-17°C ("dish" experiments). 2DG was prepared as a 1.0M stock solution in distilled water buffered at pH8 with 0.01M (final conc.) glycylglycine (gly.gly.)(13). The stock solution, which was approximately isosmotic with sea water, was diluted to desired concentration with 0.01M gly.gly. sea water at pH8. Control eggs were mixed with glucose solutions, prepared in the same manner as 2DG, or with gly.gly. sea water alone. In manometric experiments eggs were resuspended in 0.025 M gly.gly. sea water, pH 8, after the last washing. The diluent contained 100 units penicillin and 0.1 mg streptomycin ml. Egg suspensions were added to Warburg vessels from 10 to 45 minutes after fertilization, and an appropriate volume of 2DG, glucose, or 0.01 M gly.gly. sea water was added from a side arm either at 15 minutes (during temperature equilibration or just before vessels were placed in bath) or at about 105 minutes after fertilization. Oxygen uptake was measured at 19.5°C by direct method with air as the gas phase.

Results. Effect on cleavage and development. 2DG was tested at concentrations of 10^{-5} to 0.5M, and the eggs observed 40 to 60 hours, by which time all control embryos were at gastrula stage. Concentrations from 10^{-3} to 0.5M invariably arrested development, but there were variations between experiments as to the stage at which development stopped. This seemed to be due to differences in susceptibility of eggs from different animals. A summary of results of these tests, with number of experiments in parenthesis, follows: $10^{-3}M$(5) and $10^{-2}M$(7) prevented eggs from gastrulating; eggs reached early (unhatched) or swimming blastula stage. *0.1 M*(6) delayed first cleavage, but eggs eventually cleaved and some went as far as blastula stage. 0.2 *M*(4) caused a greater delay in first cleavage and generally blocked development before eggs reached blastula stage. The inhibited eggs maintained their normal appearance for several hours, but there was usually no reversal of inhibition upon washing with sea-water. Glucose at 10^{-3} to 0.2M did not retard development; however, eggs in glucose (concentrations as low as 10^{-2}M) developed somewhat abnormally at later stages. They formed blastulae and gastrulae with unusually thick walls and occasionally gastrulation was not completed.

TABLE I. Effect of 2DG and Glucose on O_2 Consumption of Eggs, in 8 Experiments.

Time of addition of sugar (min. after fertilization)	Rate O_2 uptake of treated eggs 6 hr after sugar added (as % of control)						
	2DG conc., moles/l				Glucose conc., moles/l		
	.5	.2	.1	.01	.5	.2	.1
107		7	17	38		96	106
110		24	28	71		88	99
105	16	31			91	92	
105	30	48			100	101	
15	32	54			117		
110	31	52					
15	15	37			93		
105	17	29					
15			37			91	
15				72			

Eggs in .5M 2DG cleaved only once or twice; .5M glucose did not appreciably affect rate of cleavage.

Effect on oxygen uptake: Manometric experiments were run for 7 to 10 hours, at which time the eggs were removed from flasks and their stage of development determined. Each concentration of 2DG tested (10^{-2} to 0.5M) inhibited both oxygen uptake and development. The extent of inhibition of oxygen uptake is shown in Table I. Eggs in 0.1 and 0.2M, and in other experiments 0.5M, 2DG showed no rise in rate of oxygen uptake such as that found in normally developing controls. As control rate increased, the rate for treated

eggs remained constant and eventually declined. The length of time required for the decline in rate to begin depended on concentration of 2DG. Oxygen uptake of eggs in 10^{-2}M 2DG also lags as control rate increases, but nevertheless it increases above its original level before inhibition sets in. (In one experiment the curve for 0.2M was of this type.) Glucose had essentially no effect on oxygen uptake.

Inhibition of development was about the same as in the dish experiments. Eggs in 10^{-2}M 2DG were one to 2 cleavages behind controls, which were at a late cleavage stage (about 256 cells) at the end of the experiments, while eggs in higher concentrations were more retarded.

There was a definite relationship between inhibition of oxygen uptake and inhibition of cleavage. The earlier the inhibition of oxygen uptake occurred, the greater was the retardation of cleavage.

Glucose as a counteractant to 2DG: One characteristic of competitive inhibition is that an excess of normal substrate will counteract the effect of the inhibitor. To determine whether inhibition caused by 2DG was competitive in nature, eggs were treated with mixtures of 2DG and glucose with the result that glucose almost completely counteracted the effect of 2DG on respiration (Table II). The delay in cleavage caused by 2DG was also offset by glucose. Addition of glucose after inhibition had begun, did not reverse the effect of 2DG on respiration or cleavage. In mixtures of 0.01M 2DG and 0.05M succinate, inhibition was as great as in 2DG alone; succinate did not alleviate the effect of 2DG.

Discussion. Our experiments show that 2-DG inhibits cleavage and oxygen consumption of sea-urchin eggs. Cleavage is first slowed, then totally blocked by 2DG. Since the effect of 2DG is counteracted by excess glucose, it is tentatively concluded that 2DG competitively inhibits glucose metabolism of the eggs.

Sea-urchin eggs contain enzymes both for anaerobic glycolysis and the hexose monophosphate shunt(14). Possible sites on these pathways at which glucose metabolism may be inhibited are glucose → glucose-6-phosphate,

TABLE II. Effect of Excess Glucose on Respiratory Inhibition of Developing Eggs by 2DG, in 3 Experiments.

Rate of O_2 uptake 8 hr after addition of sugars, expressed as % of control rate in sea-water	
2DG only, .01M	2DG .01M + glucose .1M
57	92
24	92
18	94

since egg hexokinase phosphorylates both glucose and 2DG(15); glucose-6-phosphate → fructose-6-phosphate(16); and glucose-6-phosphate → 6-phosphogluconic acid, which is the first reaction of the shunt. The latter site is suggested, although it has not been demonstrated to be inhibited by 2DG, because glucose metabolism in sea-urchin eggs may proceed largely through the shunt(17,18).

It is also possible that phosphorylation of 2DG slows cleavage to some extent by reducing intracellular level of adenosinetriphosphate (ATP). 2DG-6-phosphate apparently is not metabolized(19), and the phosphorylation would therefore consume ATP without eventually leading to synthesis of more ATP.

2DG should be a useful tool in the study of egg metabolism and cleavage, although for the latter purpose the high concentrations required for rapid inhibition may be disadvantageous. The delay observed with low concentrations may be due to a slow uptake of 2DG by eggs, or it may be inherent in the mechanism of inhibition.

Summary. 2DG inhibits cleavage and oxygen consumption of sea-urchin eggs. High concentrations inhibit cleavage and oxygen uptake at early stages and low concentrations inhibit at later stages of development. The effect of 2DG was counteracted by excess glucose which suggests that 2DG affects cleavage by competitively inhibiting glucose metabolism.

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Received September 14, 1959. P.S.E.B.M., 1959, v102.

Alkali-Soluble and Insoluble Collagen in Infant, Adult and Cirrhotic Livers.* (25309)

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Recent investigations emphasized the importance of the distinction between insoluble and soluble collagen, the latter being tentatively considered a precursor of insoluble collagen(1,2,3,4). The radioactive - isotope studies of Harkness indicated that alkali-soluble collagen rather than acid-soluble collagen is the true precursor(3). During evolution of experimental hepatic fibrosis produced by ethionine, the increase in alkali-soluble collagen precedes that of insoluble collagen and relative rate of increase of soluble collagen greatly exceeds that of the insoluble(5). A similar fractionation of collagen was performed, therefore, on human livers in which collagen was increasing either under physiologic circumstances, as in the infant, or under pathologic conditions, as in cirrhosis. Concentration of hydroxyproline as a measure of collagen was compared with histologic distribution of collagen and reticulum fibers.

Materials and methods. All livers were obtained at autopsy. The adult control group was derived from patients between ages 33 and 90, who died from diseases without known influence on hepatic connective tissue. The infant group comprised 7 premature newborn infants and 8 full-term infants, varying in age from 1 day to 9 months. The cirrhotic group consisted of 10 postnecrotic cirrhoses, one case

TABLE I. Alkali-Soluble and Insoluble Collagen in Infant, Adult and Cirrhotic Livers.

		Total liver		Alkali-soluble fraction			
	No. of cases	Hydroxy-proline mg %*	Collagen† mg %	Hydroxy-proline mg %	Collagen mg %	Sol. collagen as % of total %	Histological grading‡ Collagen / Reticulum
Control adult	6	.376 ±.122§	2.80	.039 ±.009	.29	10.58 ± 2.62	++ / ++
Infant	15	.155 ±.036	1.15	.037 ±.013	.27	24.10 ± 9.00	+ / +
Cirrhosis	12	1.391 ±.590	10.37	.313 ±.081	2.33	22.57 ± 5.90	++++ / +++++

* mg % of dry, defatted liver. † Collagen content calculated on the assumption that both soluble and insoluble collagen contain 13.4% hydroxyproline. ‡ Collagen and reticulum were graded histologically 1+ to 5+. § Stand. dev.

* Supported by Office of Surgeon General, Dept. of Army under Contract No. DA-49-007-MD-790.

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