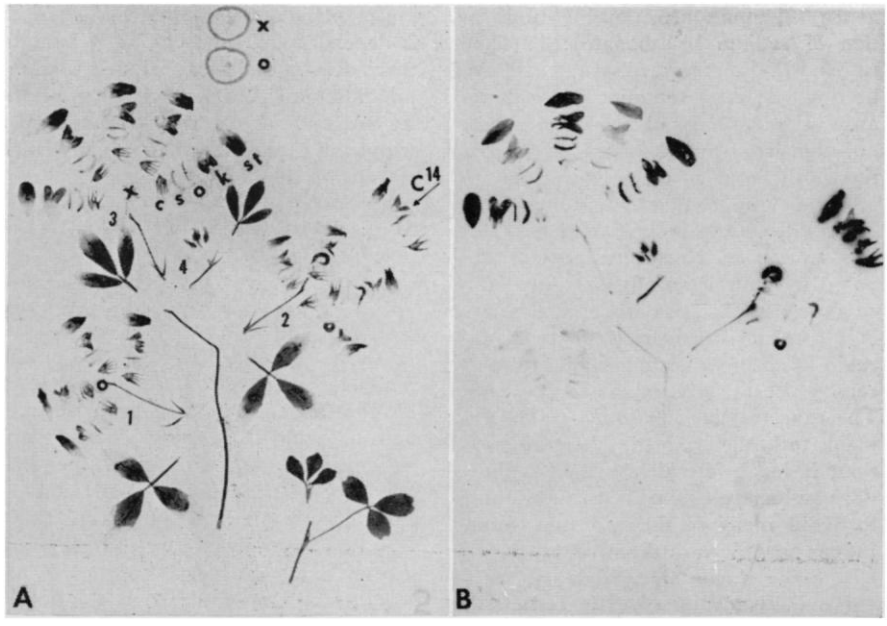

Fig. 1. Results of the experiments described in text, shown in photograph (*A*) and in autoradiogram (*B*). The point of application is indicated by the arrow. The flower parts are arranged from the outside in the following order: standard petal (*st*), wings and keel (*k*), ovary (*o*), staminal column (*s*), and calyx (*c*). On the raceme (2) with the treated flower, the pods from three of the flowers in addition to that from the treated one became radioactive, but the floral parts which had developed before the treatment did not react. One pod failed to grow. All parts of the five flowers of the upper raceme (3) which opened after the treatment became radioactive. Nectar from this raceme (3) and from the one below (1) was placed in the circles labeled *x* and *o* at the top of *A*. This nectar was also radioactive. The raceme at the lower left (1), which was in full bloom at the time of treatment, was not pollinated and became only partially radioactive. The terminal bud (4) located approximately in the center of *A* and the leaf at the lower right that developed after treatment were radioactive. Older leaves that had completed growth before treatment did not create an image on the negative. [Photographic work by W. P. Nye]

flowers on the raceme, including the treated one, were cross-pollinated by hand just before treatment. The plants were grown in a greenhouse in the absence of pollinating insects. After 6 days the plant was dissected, mounted on cardboard, pressed, and dried, and autoradiograms were prepared.

The results of the experiments, as shown by the autoradiogram (Fig. 1B) and picture (Fig. 1A), demonstrate that nectar is reabsorbed. The absorbed material is distributed primarily to growing parts of the plant, such as leaves, flowers, and pollen, but can also be found in the roots and in nectar of flowers that develop after the treatment.

Leaves which were completely developed before treatment did not become radioactive. On an adjacent raceme, flowers that had opened before treatment were much less radioactive than those that opened after treatment. However, nectar from flowers on both racemes showed images on the autoradiogram. In this case, nectar (as sucrose with C^{14}) was absorbed from a flower on one part of the plant and translocated and secreted in flowers some distance away (see Fig. 1). Similar results have been obtained with a number of other species.

In a time series test, parts of alfalfa plants adjacent to treated flowers were removed and checked for radioactivity at intervals of ½, 1, 3½, and 8 hours after treatment. These showed that reabsorption occurred within ½ hour after treatment and pollination, and that the amount of reabsorption was roughly proportional to the time interval after treatment.

On the assumption that sucrose is representative of nectar, it has been demonstrated that nectar is absorbed by nectaries as well as secreted by them. The fact that absorption occurred shortly after treatment suggests that nectar is not a static product (dissociated, so to speak, from the plant) but is in close contact with the plant system. Additional work will be needed to show whether or not nectar is also absorbed prior to pollination or senescence of the flower.

The question arises as to whether or not the nectar that is not removed by bees has any significance in the production of seed. If it does, the good production of alfalfa seed obtained by use of bees which collect mainly pollen may be partly a result of the fact that less nectar is collected. Obviously, nectar is a source of food adjacent to the developing embryos in a fertilized flower. Brink and Cooper (*3*) considered the nutrient supply to the seed to be a factor in seed failure.

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16 December 1957

Conversion of Indirect- to Direct-Reacting Bilirubin in vivo

It is now well established that the difference between indirect- and direct-reacting bilirubin lies in the fact that the latter is conjugated with glucuronic acid (*1*). Considerable evidence indicates that indirect bilirubin in high concentrations is toxic to the nervous system (*2*), particularly in the neonatal period. Kernicterus, which is found in association with high levels of indirect bilirubin in the plasma, is regarded as bilirubin encephalopathy. If it were possible to control the high levels of indirect bilirubin in hemolytic disease of the newborn and in other hyperbilirubinemias seen at this time of life, the need for exchange transfusions would no longer exist.

It is known that an enzyme, glucuronyl transferase, involving uridine diphosphate glucuronic acid is concerned in the esterification of bilirubin with glucuronic acid (*3*), an enzyme which is deficient in the liver of the newborn (*4*). In addition, there appears to be an extrahepatic mechanism for the conversion of bilirubin to its glucuronide, although the

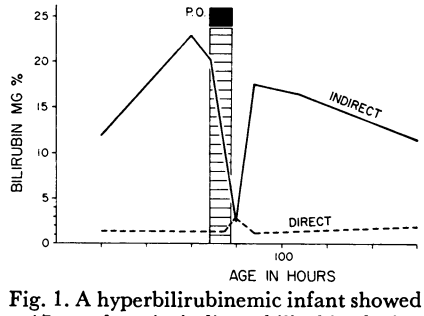

Fig. 1. A hyperbilirubinemic infant showed a 17-mg drop in indirect bilirubin during oral administration of 15 g of glucuronic acid.

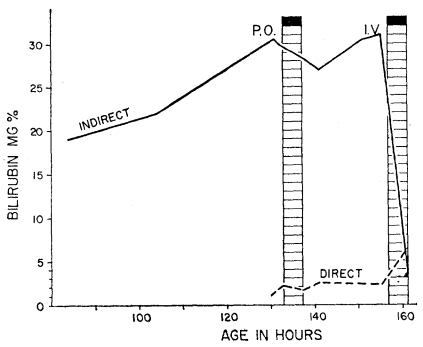

Fig. 2. A hyperbilirubinemic premature infant showed a negligible response to the oral administration of 5 g of glucuronic acid but a large decrease in indirect bilirubin during intravenous administration. Note the slight rise in the direct-reacting pigment during intravenous administration.

details of the reaction are not known at present (*5*). Even though there are no known pathways for the utilization of free glucuronic acid in glucuronide syntheses, there is some experimental evidence that such a pathway may exist. Attempts have been made to promote glucuronide conjugation in man (*6*) and animals (*7*) with glucuronic acid or glucuronolactone, but the results of these investigations are contradictory and inconsistent. Nevertheless, it seemed desirable to make the attempt to control indirect hyperbilirubinemia in the newborn by the administration of glucoronic acid.

This attempt has met with a considerable measure of success. Twenty-eight infants with hyperbilirubinemia have been treated to date by oral administration of glucuronic acid. In 16 of these a striking fall in the concentration of indirect bilirubin was observed, as is illustrated in the accompanying graph (Fig. 1), with an equally striking rebound within a few hours after the discontinuance of the conjugating agent. A simultaneous but less marked rise in direct bilirubin has been observed in some, but not in all instances. Of the 16 patients who responded, 10 were infants with erythroblastosis, the remainder being instances of physiological hyperbilirubinemia in which the concentration of indirect bilirubin exceeded 20 mg/100 ml. Of the patients who failed to respond to the oral administration of glucuronic acid, one, a premature infant, subsequently responded to intravenous administration of the acid (Fig. 2). Administration of glucuronic acid by the oral route produced a mild-to-moderate amount of watery diarrhea and acidosis in most of the infants treated. This prompted us to pursue intravenous glucuronic acid therapy further. We have now treated 14 additional infants by intravenous administration of glucuronic acid (and simultaneous oral administration of sodium bicarbonate); 12 have responded. No untoward symptoms have been noted during the injection or thereafter. The urinary excretion of direct bilirubin has been measured in two patients. In both, a three- to fivefold increase in the conjugated product was noted during and immediately after administration of glucuronic acid as opposed to control periods.

It appears that glucuronic acid per se is successful in lowering indirect bilirubin levels in serum in a significant percentage of hyperbilirubinemic patients. The exact mechanism of this action remains to be determined. These observations have obvious therapeutic implications and suggest that it may be possible to avoid many of the exchange transfusions now given in the neonatal period (*8*). Since glucuronic acid is not without toxicity, caution is required in its clinical use.

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26 November 1957

A Theory of Active State Mechanisms in Isometric Muscular Contraction

This report (*1*) presents in outline a formulation of the interaction of the contractile component and the series elastic component of isometrically contracting muscle. In general our theory is like that of Hill (*2*), but our assumptions and other details are significantly different. Our first assumption is that the basic property of the active state of the contractile component, the capacity to bear a load [given maximally by P_0 (g), the maximal force in isometric tetanus], is dependent on time (Hill's P_0 was independent of time). In simple, first approximation it is assumed that after onset this parameter rises exponentially with time constant α_1 (sec). Thus Hill's classic force-velocity relation,

$$(P + a)(v + b) = b(P_0 + a), \qquad (1)$$

is altered to read

$$(P + a)(v + b) = b[P_0(1 - e^{-t/\alpha_1}) + a]. \qquad (2)$$

In these equations: P(g) = force of the muscle; v(cm/sec) = shortening speed of the contractile component; t(sec) = time; and a(g) and b(cm/sec) are constants. We normalize Eq. 2, and all subsequent ones, so that quantities having dimensions of force are measured relative to $P_0 = 1$. Thus, by defining $p = P/P_0$ and $a_0 = a/P_0$, Eq. 2 becomes

$$(p + a_0)(v + b) = b(1 - e^{-t/\alpha_1} + a_0). \qquad (2a)$$

Our second assumption is based on the now well-known (*3–6*) nonlinear elasticity of the series elastic component [Hill (*2*) assumed a linear elasticity], and we express this by

$$p = f(e^{s/\lambda} - 1), \qquad (3)$$

in which s(cm) = the strain; p = the normalized stress; and f and λ (cm) are constants. By proper choice of f and λ, this equation can be made to fit the data of each of the previously mentioned studies (*3–6*) with remarkable accuracy.

Now, by differentiation of Eq. 3, the velocity of strain of the series elastic structure in an isometric contraction is

$$v = \frac{ds}{dt} = \frac{\lambda}{p + f}\frac{dp}{dt}, \qquad (4)$$

which, on substitution in Eq. 2a, yields

$$\frac{dp}{dt} = \frac{b}{\lambda}\left(\frac{p + f}{p + a_0}\right)(1 - e^{-t/\alpha_1} - p). \qquad (5)$$

This equation cannot be explicitly integrated and must therefore be solved numerically. However, this is required over only a very short initial time interval, for, as will be seen later, α_1 is very small compared with the total contraction period of a tetanus (or even of a twitch), and so for times greater than about $5\alpha_1$, Eq. 5 reduces to

$$\frac{dp}{dt} = \frac{b}{\lambda}\left(\frac{p + f}{p + a_0}\right)(1 - p), \qquad (6)$$

for which the explicit integral is

$$\frac{2bt}{\lambda} = \ln\frac{f}{(f + p)(1 - p)} + \frac{2a_0 + 1 - f}{1 + f}\ln\frac{f + p}{f(1 - p)}. \qquad (7)$$

For computation of Eqs. 5, 6, and 7 we use, for the frog sartorius, here studied at 20°C, the standard average values: