existing lysine deficiency and (ii) pharmacodynamic properties of lysine. In an attempt to evaluate what influence the ingestion of lysine has on human physiology, we have undertaken a number of studies. The one reported here concerns the influence on gastric secretion.

Twenty-one adult volunteers were given two test meals each, the first consisting of bread and water and the second of an equal amount of bread and water with 5 g of lysine monohydrochloride added. The two meals were separated by an interval of 1 week. Fractional gastric analysis followed each test meal, and the amounts of free hydrochloric acid and pepsin were determined in samples taken every 15 minutes for 1 hour. The methods used for these analyses are those described by Hawk, Oser, and Summerson (*2*).

The significance of all figures obtained by analysis was determined by $t$ and $P$ values obtained from the formula

$$t = \frac{M_1 - M_2}{\sqrt{(\text{S.E.}_1)^2 + (\text{S.E.}_2)^2}}$$

Values of $P$ are charted from Fischer's table. Values are considered significant when the $P$ value is less than .01.

Eighteen of the 21 subjects showed significant differences of gastric secretions after the two test meals. Whenever the addition of lysine was accompanied by an alteration, the change was an increase. The mean values of all data are given in Table 1. The incidence of effects on pepsin and hydrochloric acid secretion together or separately is as follows: increased pepsin and increased HCl, 12 subjects; increased pepsin but no increase of HCl, three subjects; no increase of pepsin but increased HCl, three subjects; no increase of pepsin or HCl, three subjects.

The addition of 5 g of lysine monohydrochloride to a bread and water test meal significantly increased gastric secretion of hydrochloric acid and pepsin, or both, in 18 of 21 subjects studied. Further work is being done to explain and localize this action. It may be of some significance that previous work has developed evidence that amino acids, either by action in the intestinal tract (*3*) or when given intravenously by systemic action (*4*), promote gastric secretion. The present study may indicate that part of the effect of lysine given as a nutritional supplement results from the stimulation of gastric secretion. This, of course, does not preclude or compete with any importance which lysine may have as a participant in anabolic processes (*5*).

Table 1. Effect of addition of lysine monohydrochloride to diet.

| Time (min) | Mean values (units) | | Difference | |
|---|---|---|---|---|
| | No lysine | With lysine | Mean | S.E.* |
| | *Hydrochloric acid* | | | |
| 15 | 3.7 | 2.5 | −1.1 | 1.12 |
| 30 | 13.4 | 24.2 | 10.8 | 5.95 |
| 60 | 13.7 | 33.7 | 20 | 2.07 |
| | *Pepsin* | | | |
| 15 | −48 | 5 | 53 | 2.31 |
| 30 | 1 | 102 | 101 | 5.35 |
| 60 | 30 | 169 | 139 | 8.51 |

* The standard error was determined from the formula $\sqrt{\frac{(\text{SD}_1)^2}{N} + \frac{(\text{SD}_2)^2}{N}}$

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## Delayed Pain as a Peripheral Sensory Pathway

The existence of "second pain" as a "genuine sensory phenomenon" was recently questioned (*1*). There was also the more interesting implication that a delayed type of "C-fiber pain" does not exist at all as a separate pain sensation. "Double pain" can be aroused by an appropriate noxious stimulus if it is applied to the skin far enough from the central nervous system (*2, 3*). The "second" or slower pain is felt at a distinct time interval after the first or fast component, and it is usually qualitatively different from the first, being more a diffuse ache than a sharp prick, more unpleasant, longer lasting, and like the pain that is aroused from deeper structures below the skin (*4*). Landau and Bishop (*4*) point out that this delayed type of pain has a greater threshold for most types of stimuli than does sharp pricking pain, although its threshold is lower for certain stimuli. They also present evidence (evidently missed by Jones) that the delayed pain sensation can be masked by the faster sharp pain; this makes it difficult to detect double pains under certain conditions of stimulation.

I suggest, therefore, that what the experimental work reported by Jones (*1*) amounts to is a careful description of some conditions of stimulation in which second pain is not demonstrable. For example, threshold electric stimuli will never elicit second pain, even in "480 separate determinations," if those receptors and nerve fibers subserving fast pain are electrically more excitable than those subserving slow pain sensations. The more slowly conducting, amyelinated C fibers, as well as their receptor endings (*4*), have a higher electric threshold than any other nerve-fiber group.

Jones concluded that second pain, when it does appear, is an experimental "artifact" rather than a "genuine sensory phenomenon." These distinctions have little meaning without careful definition for this context. Jones' main contention would appear to be that if a noxious stimulus were delivered at the same instant to any of the receptors that subserve pain, only a fast single, and not a double, pain could be produced—that is, that there is only one type of pain-sensory system; but her negative results do not prove this. If it is difficult [although not impossible (*4*)] to find a stimulus which excites the receptors that subserve slow pain without also exciting other types of receptors, this difficulty cannot be used as proof that slow pain is not a "genuine" and distinct sensory modality. More definitive evidence on this point must be sought from other experimental procedures. It is important, therefore, to analyze some of the criticisms (*1, 5*) of these other, more positive lines of evidence.

Although the total reaction time for perception of pain is obviously not a measure of conduction time, the *increase* in reaction time found when the stimulus is moved to a more distal point (*2*) can be a measure of conduction velocity in the sensory fibers. Jones dismisses such evidence by citing a more recent report (*6*) to show that the increase in reaction time with a more distal stimulus is small and relatively insignificant. But this negative finding concerned touch sensations, not pain (*7*). If the impulses that are responsible for touch sensation are transmitted in group A fibers of even the small variety, for example, at 10 m/sec, then the increase in reaction time that could be expected when the stimulus is moved 50 cm distally would be only 0.05 second, and difficult to detect. On the other hand, if delayed pain is mediated by C fibers, at 1 m/sec, the difference in reaction time would be 0.5 second. This interval, which is easily detectable, was found to be the actual difference in reaction time to slow pain for the conditions stated (*2*).

Although all cutaneous senses show an increasing delay in perception during

compression ischemia (*1, 8*), the delay in perception of pain tends to increase rather suddenly and at a time when touch sensibility is lost (*9*). Zotterman explained this plausibly as follows: fast pain impulses are transmitted along with touch impulses in the A-fiber group, and since the A group ceases functioning before the C group during ischemia, one is left rather suddenly with only slowly conducted, C-fiber, "second-pain impulses." There is general agreement (*3, 4, 8–10*) that after the loss of other sensations has occurred during compression block, a slow, very unpleasant, aching pain sensation can still be aroused or even becomes more evident or "unmasked."

This, and the reverse finding with partial procaine block (that is, abolition of the slow, aching pain with retention of the sharp, pricking pain), also means that the functional pathways for the two types of pain are separable. The suggestion (see *11*) that various pains are simply the result of sufficiently intense activity in any sensory pathway cannot have general validity, since impulses at high frequencies in large numbers of the larger myelinated fibers in a nerve trunk lead only to a tingling sensation without pain (*12*). The common factor with intense tissue stimulation of any kind is tissue damage, and apparently this is the stimulus to which the receptor endings of the "pain fiber" systems are especially sensitive (see also *4*).

The work of the Oxford group (*5, 8*) is cited by Jones to show that the earlier evidence (*3*) for the separability of fast and slow pain, based on the order of loss of sensation during nerve blocks, now appears to have been unreliable. But a careful examination of these papers reveals that this conclusion may not be warranted, although admittedly their general summary statements apparently agree with Jones' evaluation. Sinclair and Hinshaw (*8*) state that they did in fact find statistically significant differences in the order of sensory loss between procaine block and compression block of a given nerve. They also point out some of the factors which could have accounted for the variability of results in their human sensory experiments, when compared with the consistent orders of fiber sizes blocked in animal experiments. Other factors that may account for this variability have been suggested (*4*), to which one may also add that of variation in available tissue buffers which neutralize procaine hydrochloride to the more penetrative free alkaloid. Consistent, clear-cut orders of sensory losses can be obtained by using more dilute procaine solutions on smaller peripheral nerves (*4*) and in spinal anesthesia (*13*), where the sheaths of the spinal rootlets are also uniformly thin.

The apparent failure of histological specificities in receptor end-organs to account for the different sensory modalities found in a given area of skin (*14*) simply shows that the common textbook conception of the correlation between four types of receptor end-organs and four cutaneous sensory modalities is too rigid. Surely, physiological specificities for responses to different stimuli may still exist, even among apparently uniform receptor nerve endings, based on structural and chemical specificities that are not evident with present histological techniques.

Thus, the simplest and best supported hypothesis available, among those hypotheses that have been proposed for relating the pain modalities to the sensory nerve fibers (and one still held fairly widely among physiologists) maintains (i) that pain in general is mediated predominantly by small myelinated (delta) and amyelinated (C) sensory fiber groups and (ii) that, in the skin tissues, fast, sharp pain is mediated predominantly by the first fiber group, while the slow, more unpleasant pain is mediated predominantly by the second. Sensations other than pain are, of course, not necessarily excluded from being represented in these fiber groups (*3, 8*), nor is the possibility excluded that the total pattern of impulses arriving in different peripheral pathways may affect the nature of the subjective sensation that results. In deeper tissues (periosteum, muscle sheaths, and so forth) the more unpleasant pain, without sharpness, is apparently mediated by both groups of small fibers, although even here the C fibers probably are responsible for the slower, more penetrating, longer-lasting component (*4*).

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In my original paper (*1*), the conclusions presented were based on the evidence. Detailed consideration of each bit was impossible because of limitation of space. The foregoing discussion by Libet adds nothing new. It is a restatement of a selected portion of older literature, most of it useless because of failure to control stimulation and because of ignorance of proper conditions of judgment and psychophysical methods (*2*).

The loose accusation that I missed the point that "fast pain" may mask "slow pain" (*3*) is unjust, for it is *specifically* referred to in paragraph eight (*1*). It is a mere assumption, without a shred of evidence to support it, invented to bolster a popular hypothesis. Even the authors could not get most of their "unprejudiced" subjects to feel "second pain."

Space permits comment on only two specific points. The statement that "slow pain" has a higher threshold than "fast pain" is, again, an assumption. It is a conclusion drawn from the fact that abnormally functioning tissue (*4*) has a higher threshold than normal tissue, and so do C fibers. The connection between the two is *at present* without evidence. In my experiment, suprathreshold mechanical stimuli were used without arousing "slow pain." Suprathreshold electric stimuli create serious problems.

Second, Libet's emphasis on reaction time is unfortunate. None of the studies meet the minimum requirements for work in this field (*5*). The negative finding with respect to touch (*6*), the result of much more careful study than the earlier studies on pain, was cited to instill caution in dealing with pain, where the time of action of the stimulus is indeterminate. A difference of 50 milliseconds is, incidentally, readily demonstrable with proper techniques.

Dependence on results of ischemia or blocking, or both, is unwise (*4*). Also, plots of decrease in impulse amplitude and increase in latency against duration of ischemia show *smooth* curves (*7*). Likewise, no correlation was found between conduction time and blocking time [for various touch fibers, and presumably also for others (*8*)].

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## An in vitro Effect of Vitamin D on Citrate Oxidation by Kidney Mitochondria

The possibility that an *in vitro* effect of vitamin D may be demonstrable appears evident from the effects on citrate metabolism which have been reviewed in recent publications (*1, 2*). In these it was reported that additions of vitamin D to rachitogenic and nonrachitogenic diets reduced the oxidation of citrate by kidney homogenates and mitochondria, thereby accounting for an increase in the citrate content of certain tissues and an increase in the citrate excretion in urine. Lately, we have succeeded in demonstrating a reduction in citrate oxidation by kidney mitochondria when vitamin D was added *in vitro*.

Young, male rats of the Sprague-Dawley strain were made vitamin-D deficient by the feeding of either a rachitogenic or a nonrachitogenic diet as described earlier (*1*). They were killed by decapitation, and kidney mitochondria were prepared, essentially by the method of Schneider (*3*).

The oxidations were carried out in a Warburg apparatus at 30°C with air as the gas phase. The incubation mixture, 3 ml in volume, contained 40 μmole of phosphate buffer (*p*H 7.3), 20 μmole of $MgCl_2$, 6 μmole of adenosine triphosphate, 0.08 μmole of cytochrome *c*, isotonic sucrose, and the indicated additions. Forty micromoles of glucose and excess hexokinase (Sigma) were added from the side arm of the flasks to prevent limitation of oxidation by a lack of phosphate acceptor (*4*). All substrates were added in amounts of 15 μmole, except citrate and succinate, which were added at 45 μmole, and oxalacetate, which was added at 10 μmole with an addition of 15 μmole of pyruvate.

When desired, the flask contents, prior to and following incubation, were deproteinized with 10-percent trichloroacetic acid. The filtrates, for the calculation of P/O ratios, were analyzed for P by the method of Lowry and Lopez (*5*). Citrate was determined by the method of Speck, Moulder, and Evans (*6*), and keto acids by the method of Friedmann and Haugen (*7*). Vitamin D and other compounds tested for their effect were added in propylene glycol or occasionally in the ethanol-serum albumin-phosphate buffer suspension of Nason and Lehman (*8*). In all cases, only 0.05 ml of each of these preparations was added to the contents of a flask. Control flasks received an equivalent amount of appropriate carrier.

Table 1. The *in vitro* effect of vitamin D on the oxidation of various substrates by kidney mitochondria.

| Substrate | Without vitamin D (μl $O_2$) | With vitamin D* (μl $O_2$) | Decrease (%) |
|---|---|---|---|
| Citrate | 85 | 32 | 62 |
| Isocitrate | 81 | 28 | 65 |
| α-Ketoglutarate | 75 | 80 | 0 |
| Glutamate | 69 | 55 | 20 |
| Succinate | 115 | 117 | 0 |
| β-Hydroxybutyrate | 35 | 31 | 11 |
| Pyruvate plus oxalacetate | 88 | 90 | 0 |

* One hundred and twenty-five micrograms of vitamin $D_2$ in 0.05 ml of propylene glycol per flask; other flasks received 0.05 ml of propylene glycol alone; 0.7 mg of mitochondrial nitrogen was added to each flask.

The comparative effect of vitamin D on the oxidation of various substrates (Table 1) clearly shows that vitamin D had a pronounced effect on citrate and isocitrate oxidation. Its effect on glutamate oxidation was small, but significant, while on the oxidation of α-ketoglutarate, succinate, β-hydroxybutyrate, and on pyruvate in the presence of oxalacetate, it had little or no effect. It is interesting to note that only the triphosphopyridine nucleotide systems are affected if one considers glutamate oxidized by both tri- and diphosphopyridine nucleotide pathways.

Table 2 illustrates the action of vitamin D in reducing citrate oxidation and α-ketoglutarate production while not affecting coupled phosphorylation efficiency to any degree.

In experiments not shown here, vitamins $D_2$ and $D_3$, when added *in vitro* in either of the carrier systems used, were equally active in reducing citrate oxidation, while equal quantities of 7-dehydrocholesterol, ergosterol, $\Delta^7$-cholestenol, and cholesterol were inactive. However, it should be noted that in view of the different solubility characteristics of the sterols as compared with vitamin D, the possibility that they did not enter the mitochondria cannot be ruled out.

Table 2. The *in vitro* effect of vitamin D on the accumulation of α-ketoglutarate during citrate oxidation. The values represent an average of at least six determinations.

| | Citrate oxidized (μg) | Oxygen consumed (μl $O_2$) | α-Ketoglutarate accumulated (μg) | P/O |
|---|---|---|---|---|
| Without vitamin D | 630 | 83 | 14.4 | 3.0 |
| With vitamin $D_2$* | 180 | 46 | 8.5 | 2.7 |

* One hundred and twenty-five micrograms of vitamin $D_2$ in 0.05 ml of propylene glycol per flask; other flasks received only 0.05 ml of glycol; 0.7 mg of mitochondrial nitrogen was added per flask, and the oxidation was continued for 10 minutes.

The possibility that the resulting accumulation of citrate in kidney, and possibly in other tissues, may be an important factor in calcium transport and deposition appears increasingly alluring. Studies on these phases are in progress, especially with relation to specific enzyme systems.


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## Insect Nutrition and Metabolism of Sterols

The importance of cholesterol and related steroids in insect nutrition was first demonstrated in 1935 (*1*), and it is now well established that insects in general require a dietary sterol (*2*). The insects' nutritional requirement for sterols is indicative of an inability to synthesize the steroid nucleus, at least in physiologically adequate amounts. This characteristic is in direct contrast to that of the higher animals, in which steroids are apparently synthesized from simple com-