Earlier reported depressant \{beta-alanine (1, 2) and 1-glutamine (2, 3)\}, augmentary \{1-cystine (2, 3) and possibly cysteine (2)\} and indifferent \{1-histidine (2, 3)\} effects of parenterally administered amino-acids on the titres of circulating antibodies, whether induced following repeated/consecutive, multiple—antigenic stimulation (1, 3) or after once exposure to a single ‘antigen’ (2) were analysed further to elucidate some facets of the mechanisms of these effects. The earlier studies (2) showed that in the controls the titres of circulating antibodies against Typhoid ‘H’ antigen on the 21st and 28th day, after a single antigenic stimulus was of the order of 426 ± 106 and 16 ± 11 respectively. With concurrently administered beta-alanine, cystine, cysteine, histidine and glutamine, these values on the 21st day were 133 ± 46, 746 ± 489, 5640 ± 2686, 266 ± 269, and 433 ± 369 respectively and on the 28th day these were 6 ± 11, 26 ± 11, 26 ± 11, 26 ± 11 and 20 ± 0 respectively.

On account of the effect of beta-alanine, cystine and cysteine being more striking, the present studies were undertaken with respect to these amino-acids to elucidate further aspects of the involved mechanism(s).

Eighteen rabbits of both sexes, weighing between 1-1.5 kg and maintained on balanced, commercial diet were divided into six groups of 3 rabbits each. One group each out of these groups received only beta-alanine, cysteine and 1-cystine subcutaneously in the doses of 50 mg/kg body weight daily for 28 days. The antibody titres against Typhoid ‘H’ antigen were determined on their sera by Widal’s agglutination technique prior to the beginning of the experiments and then at weekly intervals.

To the other groups, a single antigenic stimulus was provided by the administration of subcutaneous injection of 1 ml of the suspension of killed formalinized Salmonella typhi containing approximately 10 × 10⁵ organisms. On the 21st day, when in accordance with the earlier study (2) highest titres were expected to have been reached, the administration of the amino-acids in the aforementioned dosage daily to different groups of animals was started, after estimating the serum antibody titre against Typhoid ‘H’ antigen. A further estimation of the serum antibody titres was made on the 28th day i.e. seven days later, when in the control group as well as in the groups receiving amino-acids concurrently, the levels had settled down virtually to pre-injection levels (vide Supra).

It is obvious that the amino-acids per-se did not cause any stimulation of antibody production in the animals, whether these had no detectable titres of the antibody or had
low titres of it.

It is also strikingly clear that none of the amino-acids, irrespective of what effects these might have had on the circulating antibody titres, when administered concurrently from the very beginning (1, 3), caused any significant alteration in the levels of the residual titres on the 28th day.

Since, some of the animals of group 1 did have detectable low titres of Typhoid ‘H’ antibody at the start, it can be safely inferred that these amino-acids did not possess the capacity to stimulate either the potential or the actual antibody producing clones of cells.

Further, these amino-acids also failed to modify the ‘Catabolic’ parameters of antibody metabolism, which seemed to go at its ‘control’ pace, irrespective of which amino-acid abounded in the system.

From these observations in conjunction with those recorded earlier (2), it seems plausible that the amino-acids exert their modifying effects only on the anabolic phase of the antibody production when the cells are under a state of active stimulation by antigenic stimulus.

Further studies to substantiate this hypothesis are currently in progress in this laboratory.

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**TABLE 1. Antibody titres (Expressed in reciprocals).**

| Group 1 | Rabbit No. |
|---------|------------|
| Receiving | 1 | N | N | N |
| beta-alanine | 2 | N | N | N |

| Group 2 | Rabbit No. |
|---------|------------|
| Receiving | 1 | N | N | N |
| cysteine | 2 | N | N | N |

| Group 3 | Rabbit No. |
|---------|------------|
| Receiving | 1 | 20* | 20 |
| 1-cystine | 2 | N* | 640 |

| Group 4 | Rabbit No. |
|---------|------------|
| Receiving | 1 | 20* | 320 |
| beta-alanine after 21 days of antigen injection | 3 | N* | 320 |

| Group 5 | Rabbit No. |
|---------|------------|
| Receiving | 1 | 20* | 1280 |
| Cysteine after 21 days of antigen injection | 2 | 20* | 640 |

| Group 6 | Rabbit No. |
|---------|------------|
| Receiving | 1 | N* | 160 |
| 1-cystine after 21 days of antigen injection | 2 | N* | 320 |

N : Not detected.

* : Time when antigenic stimulus was administered.
A SIMPLE QUANTITATIVE METHOD FOR THE EVALUATION OF PHYSICAL DEPENDENCE LIABILITY OF MORPHINE IN MICE

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Previously, we reported that a continuous infusion of morphine hydrochloride into mice either subcutaneously or intravenously could establish “acute tolerance” to the analgesic effect of this alkaloid within a few hours, confirming the observation by Cox et al. (1), and also obtained some evidences suggesting the similarity of the tolerance produced in this way to that routinely acquired by repeated administration (2).

On considering the well known intimate relationship of tolerance with physical dependence, therefore, it would be the natural extension of our research to examine if the mice which developed distinct tolerance to morphine after a short-term infusion, had indeed become physically dependent on this alkaloid. In the same animal species implanted with morphine pellet, Way et al. (3) have recently succeeded in quantifying the intensity of developed physical dependence by estimating the dose of naloxone to precipitate withdrawal jumping syndrome. In the experiment reported here, thus, we adopted the jumping syndrome and the loss of body weight as the index of abstinence syndrome aiming at the evaluation of the intensity of physical dependence after continuous infusion of morphine in mice.

Adult male mice (dd-strain) weighing 20±0.5 g were used. The animal was fixed in a plastic box specially designed for this experiment and the solution of morphine hydrochloride (10 mg/ml) in saline was infused subcutaneously or intravenously at the rate of 35 mg/kg/hour using a continuous infusion apparatus (Natsume KN-204) which was modified so as to treat ten animals at one time. The details of the experimental condition applied for the present study have been reported previously (2). The mice which received saline instead of morphine solution served as control. After infusion for 1, 3, 5, 7 or 10 hours, the mouse was discharged from the box, weighed and transferred onto a circular platform (30 cm in diameter and 45 cm in height). The changes in body weight at intervals of 30 minutes and the behaviour were recorded for the next three hours. Ten mg/kg